National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

UNIVERSITY OF ALBERTA

POTENTIAL USE OF ASPARTAME IN LIQUID PHARMACEUTICLAL DOSAGE FORMS

BY

SANYUDE SANYUDE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR A DEGREE OF
DOCTOR OF PHILOSOPHY
IN
PHARMACEUTICAL SCIENCES (PHARMACEUTICS)

Faculty of Pharmacy and Pharmaceutical Sciences

EDMONTON, ALBERTA
Spring, 1991

National Library
of Canada

Bibliothèque nationale
du Canada

Canadian Theses Service

Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-66823-7

Canadä

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR SANYUDE SANYUDE

TITLE OF THESIS POTENTIAL USE OF ASPARTAME IN LIQUID PHARMACEUTICAL DOSAGE FORMS

DATE DEGREE AWARDED 1991

PERMISSION IS HEREBY GRANTED TO THE UNIVERSITY OF ALBERTA LIBRARY TO REPRODUCE SINGLE COPIES OF THIS THESIS AND TO LEND OR SELL SUCH COPIES FOR PRIVATE, SCHOLARLY OR SCIENTIFIC RESEARCH PURPOSES ONLY.

THE AUTHOR RESERVES OTHER PUBLICATION RIGHTS, AND NEITHER THE THESIS NOR EXTENSIVE EXTRACTS FROM IT MAY BE PRINTED OR OTHERWISE REPRODUCED WITHOUT THE AUTHOR'S WRITTEN PERMISSION.

(SIGNED) S Sanyude

PERMANENT ADDRESS:
Masunda Village
Nyamandi School
P.O. Gutu, Zimbabwe

Date: April 14, 1991

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED **THE POTENTIAL USE OF ASPARTAME IN LIQUID PHARMACEUTICAL DOSAGE FORMS** SUBMITTED BY **SANYUDE SANYUDE** IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **DOCTOR OF PHILOSOPHY IN PHARMACEUTICAL SCIENCES (PHARMACEUTICS).**

R.A Locock

Dr. R.A. Locock

Dr. L.A. Pagliaro

Fak. Jamali

Dr. F. Jamali

Glen B. Baker

Dr. G.B. Baker

Dr. R. Foster

Dr. L.I. Wiebe

A. G. Mitchell

Dr. A.G. Mitchell

Date: April 9/90

DEDICATION

THIS THESIS IS DEDICATED TO MY LATE FATHER, SANYUDE CHAMWADADE, FOR THE SPIRITUAL INSPIRATION; MY LATE GRANDMOTHER, MBUYA VAMACHASI, FOR THE LOVE SHE INSTILLED IN MY HEART; MY MOTHER, NZWISISAI MASUNDA, FOR THE PATIENCE AND UNDERSTANDING; MY BROTHERS AND SISTERS, CHENGETO, VIOLET, MOSTAFF, CATHRINE, TERESA, FANUEL, ELIZABETH, AND STELLA, FOR THE FAITH THEY HAVE IN ME; MY WIFE, ZODWA, FOR THE TOLERANCE; AND MY CHILDREN, MELANA, TAKONDWA, AND NATASHA, FOR THE TIME THEY ENDURED WITHOUT THE FATHERLY LOVE IN ORDER FOR ME TO FINISH THIS WORK.

ABSTRACT

Formulation pharmacists have excluded the sweetener aspartame from oral liquid dosage forms because in solution, aspartame hydrolyzes to nonsweet compounds, N-L-α-aspartyl-L-phenylalanine, 5-benzyl-3,6-dioxo-2-piperazineacetic acid, and methanol. Factorial experimental designs were used in accelerated kinetic studies which examined the effects of several factors on the stability of aspartame in solution. Factors investigated were pH, buffer concentration, buffer species, ionic strength, solvent composition and temperature. The degradation of aspartame was followed by a stability-indicating HPLC assay. Acetaminophen solution and diazepam suspension were the model oral liquid dosage forms studied. The stability and potential use of aspartame in these two liquid dosage forms was evaluated.

Analysis of the data indicated that the effect of pH, buffer concentration, buffer species, solvent composition, and temperature significantly affected the rate of aspartame degradation. From the results it was shown that the half-life of aspartame, in solution, could be increased to at least two years by adjusting the pH to 4.3; by using minimum buffer concentration and using tartaric acid buffer or malic acid buffer; by using solvents with high dielectric constants; and by storing the solution at cool or cold temperatures.

Acknowledgements

I would like to express my gratitude to my co-supervisors, Dr. R. Locock and Dr. L. Pagliaro for the guidance and help throughout this study and for their valuable suggestions, constructive criticism and encouragement in the preparation of this thesis. I would also like to thank the members of the supervisory committee, especially Dr. F. Jamali, for the assistance during the course of the research and encouragement during difficult times; the Canadian Commonwealth Scholarship and Fellowship for the financial support; and my colleagues in Insaka Alkebu-lan for the stimulating discussions, and for their friendship and brotherly support.

TABLE OF CONTENTS

CHAPTER PAGE

1. INTRODUCTION....................................1
   - 1.1 HYPOTHESIS....................................4
   - 1.2 AIM....................................6
2. BACKGROUND....................................7
   - 2.1 HISTORICAL ASPECTS....................................7
     - 2.1.1 History in the United States............7
     - 2.1.2 Worldwide Status....................8
   - 2.2 MANUFACTURING....................................9
   - 2.3 PHYSICAL AND CHEMICAL PROPERTIES................10
     - 2.3.1 Solubility....................................12
     - 2.3.2 Taste....................................12
       - 2.3.2.1 Physiology of Taste................12
       - 2.3.2.2 Molecular Theory of Sweet Taste.....14
       - 2.3.2.3 Taste Quality and Intensity.........15
     - 2.3.3 Stability....................................16
     - 2.3.4 Shelf life....................................17
   - 2.4 TOXICITY ASSESSMENT....................................22
     - 2.4.1 The Methanol Moiety....................23
     - 2.4.2 The Aspartate Moiety....................24
     - 2.4.3 The Phenylalanine Moiety..............26
       - 2.4.3.1 Normal Subjects..............30
       - 2.4.3.2 PKU Heterozygotes............31
       - 2.4.3.3 PKU Homozygotes..............32

2.4.4 Consumer Complaints....................33
2.4.5 Concluding Remarks on Safety...........35
2.5 SELECTION OF DRUGS..............................36
2.5.1 Acetaminophen Stability...............37
2.5.2 Diazepam Stability....................39
3. EXPERIMENTAL SECTION..............................42
3.1 MATERIALS..42
3.2 METHODS..42
3.2.1 Stability of Aspartame in Buffered Aqueous Solutions....................42
3.2.1.1 Kinetic Method...................43
3.2.1.2 Analytical Method................43
3.2.1.3 Experimental Design..............45
3.2.1.4 Statistical Procedure............46
3.2.2 Stability of Aspartame in Mixed Solvent Systems.......................47
3.2.2.1 Preparation of Samples...........47
3.2.2.2 Experimental Procedure for the pH-Rate Profile...................48
3.2.3 Further Elucidation of Aspartame Degradation in Aqueous Solutions.......52
3.2.3.1 Analytical Method................52
3.2.4 Formulation of Acetaminophen Solution Dosage Form...................53
3.2.5 Compatibilty of Ingredients in Acetaminophen Solution Dosage Form.....54

3.2.6 Stability of Acetaminophen Solution Dosage Form.........................56
3.2.6.1 Experimental Design...............57
3.2.6.2 Analytical Method.................58
3.2.7 Formulation and Evaluation of Diazepam Suspension Dosage Form........60
3.2.7.1 Kinetic Method....................62
3.2.7.2 Analytical Method.................62
3.2.7.3 Rheological Evaluation of Diazepam Suspension..............64
4. RESULTS AND DISCUSSION..............................66
4.1 STABILITY OF ASPARTAME IN BUFFERED AQUEOUS SOLUTIONS......................................66
4.2 FURTHER ELUCIDATION OF THE DEGRADATION OF ASPARTAME...................................83
4.2.1 Specific and General Acid-Base Catalysis..............................90
4.2.2 Catalytic Effect of Buffers............94
4.2.3 The Salt Effect........................99
4.2.4 Effect of Temperature on Buffer pH....102
4.3 STABILITY OF ASPARTAME IN MIXED SOLVENT SYSTEMS..............................105
4.4 COMPATIBILITY OF INGREDIENTS IN ACETAMINOPHEN SOLUTION DOSAGE FORM..............118
4.5 EVALUATION OF THE ACETAMINOPHEN SOLUTION DOSAGE FORM...........................120

4.6 EVALUATION OF THE DIAZEPAM SUSPENSION DOSAGE FORM........126
4.7 SHELF LIFE........135
5. SUMMARY AND CONCLUSIONS........137
BIBLIOGRAPHY........140
APPENDIX........160

LIST OF TABLES

TABLE PAGE

1.1 Expressions of Half Lives and Shelf Lives of the Zero-, First-, and Second-Order Reactions....19

3.1 Calculation Matrix for a 2^3 Factorial Design....49

3.2 Dielectric Constants of Solvents at Different Temperatures....50

3.3 Chemical Composition of the Hydro-Organic Mixtures at Dielectric constant Values of 65, 55, and 45....51

3.4 Acetaminophen Solution Dosage Form....55

3.5 Matrix of a Modified 2^4 Factorial Central Composite Experimental Design....59

3.6 Diazepam Suspension Dosage Form....61

4.1 Validation of Aspartame Analysis by Peak-Area Ratio Measurement....68

4.2 Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Acetic Acid Buffer Solutions at 60°C....72

4.3 Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Acetic Acid Buffer Solutions at 50°C....73

4.4 Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Acetic Acid Buffer Solutions at 40°C....74

4.5 Changes in pK_a as Temperature Increase....104

4.6 Stability of Aspartame in Solvent Systems with Different Dielectric Constants....109

4.7 Activation Energy of the Decomposition of

Aspartame in Water-Alcohol Solutions............115

4.8 Results of Difference Ultraviolet-Visible Absorption Spectra of Ingredients of Acetaminophen Solution Dosage Form..............119

4.9 Result of Aspartame Degradation for a Modified 2^4 Factorial Central Composite Experimental Design..............................122

4.10 Analysis of Variance for the 2^4 Factorial Part of the Experiment. The Effect of pH, Buffer Concentration, Solvent Composition, and Temperature on the Degradation of Aspartame in Acetaminophen Solution Dosage Form..............123

4.11 The Effect of pH on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form..124

4.12 The Effect of Buffer Concentration on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form..............................124

4.13 The Effect of Solvent Composition on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form..............................125

4.14 The Effect of Temperature on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form..125

4.15 Result of Acetaminophen Degradation for a Modified 2^4 Factorial Central Composite Experimental Design..............................127

4.16 Analysis of Variance for the 2^4 Factorial Part of the Experiment. The Effect of pH, Buffer Concentration, Solvent Composition, and Temperature on the Degradation of Acetaminophen in Acetaminophen Solution Dosage Form..128

4.17 Results of Aspartame Degradation in Diazepam Suspension..............................131

4.18 Concentration of Diazepam in Suspensions Stored at 5°C and 35°C..........................132

LIST OF FIGURES

FIGURES PAGE

2.1 Chemical Structure of Aspartame....................11

2.2 Principal Conversion Products of Aspartame.......18

2.3 Pathway of Catecholamine Synthesis...............27

2.4 Hydrolysis of Acetaminophen.......................38

2.5 Hydrolysis of Diazepam............................40

4.1 Chromatogram of a Sample with Aspartame and its Degradation Products.....................67

4.2 Standard Calibration Curve of Aspartame..........69

4.3 Degradation of Aspartame in Malic Acid Buffer at 50°C Exhibiting Pseudo First-Order Kinetics...70

4.4 The Effect of pH, Ionic Strength (B), and Buffer Concentration (C) on the Degradation Rate of Aspartame in (a) Acetic Acid, (b) Citric Acid, (c) Malic Acid, and (d) Tartaric Acid Buffer Solutions at 60°C.......76

4.5 The Effect of Ionic Strength, pH (A), and Buffer Concentration (C) on the Degradation Rate of Aspartame in (a) Acetic Acid, (b) Citric Acid, (c) Malic Acid, and (d) Tartaric Acid Buffer Solutions at 60°C.......77

4.6 The Effect of Buffer Concentration, pH (A), and Ionic Strength (B) on the Degradation Rate of Aspartame in (a) Acetic Acid, (b) Citric Acid, (c) Malic Acid, and (d) Tartaric Acid Buffer Solutions at 60°C.......78

4.7 Arrhenius Plot for the Degradation of Aspartame in 0.1 M Malic Acid Buffer at pH 3.0............80

4.8 Degradation of Aspartame and Formation of Aspartylphenylalanine and Diketopiperazine in 0.05 M Malic Acid Buffer at pH 5.3 and 60°C...84

4.9 Degradation of Aspartame and Formation of Aspartylphenylalanine and Diketopiperazine in 0.05 M Malic Acid Buffer at pH 4.3 and 60°C...85

4.10 Degradation of Aspartame and Formation of

Aspartylphenylalanine and Diketopiperazine in 0.05 M Malic Acid Buffer at pH 3.0 and 60°C..86

4.11 Degradation of Aspartylphenylalanine and Formation of Diketopiperazine and Phenylalanine in 0.05 M Malic Acid Buffer, at pH 5.3 and 60°C................................87

4.12 Degradation of Aspartylphenylalanine and Formation of Diketopiperazine and Phenylalanine in 0.05 M Malic Acid Buffer at pH 4.3 and 60°C................................88

4.13 Degradation of Aspartylphenylalanine and Formation of Diketopiperazine and Phenylalanine in 0.05 M Malic Acid Buffer at pH 3.0 and 60°C................................89

4.14 Pseudo First-Order Degradation of Aspartame in 55.2% Ethanol-Water Mixture at Apparent pH 4.5 and 50°C.................................106

4.15 Pseudo First-Order Degradation of Aspartame in 63.8% Methanol-Water Mixture at Apparent pH 4.5 and 50°C.................................107

4.16 Pseudo First-Order Degradation of Aspartame in 78.5% Glycerol-Water Mixture at Apparent pH 4.5 and 50°C.................................108

4.17 Degradation of Aspartame in Ethanol-Water Solvent Mixtures with Different Dielectric Constant Values of 45, 55, and 65...............110

4.18 Degradation of Aspartame in Methanol-Water Solvent Mixtures with Different Dielectric Constant Values of 45, 55, and 65...............111

4.19 Degradation of Aspartame in Glycerol-Water Solvent Mixtures with Different Dielectric Constant Values of 45, 55, and 65...............112

4.20 The pH-Rate Profile for Aspartame Degradation at 60°C in 45.9% Ethanol-Water Solvent Mixture, 24.7% Ethanol-Water Solvent Mixture, and Water.......................................117

4.21 Rheogram of Diazepam Suspension, Before Shaking, Showing Thixotropy.......................133

4.22 Rheogram of Diazepam Suspension, After Shaking, Showing Thixotropy........................134

List of Abbreviations

ADI	Acceptable Daily Intake
APAP	Acetaminophen
APM	Aspartame
C_A	Total Buffer Concentration
D	Dielectric Constant
DKP	Diketopiperazine

1. INTRODUCTION

Oral liquid pharmaceuticals are used because they are easy to administer to individuals, such as pediatric patients, geriatric patients and patients with nasogastric tubes, who have difficulty in swallowing solid dosage forms (1). They are usually more bioavailable than solid dosage forms. When a drug is administered in an oral solution dosage form, the drug is immediately available for absorption from the gastrointestinal tract, and, in most cases, it is more rapidly and efficiently absorbed than from a solid dosage form. The bioavailability of drugs is assumed to decrease in the following order: solutions > suspensions > capsules > compressed tablets > coated tablets. However, many other factors besides particle size can affect the rate and extent of drug absorption, and this order of relative bioavailability does not always hold (2). For example, Hirst and Kay (3) showed that the same dose of thioridazine given either as a solution in a syrup form or formulated as oral suspension gave significantly lower serum levels of the drug when formulated in solution.

The suspension is often selected as a pharmaceutical dosage form when the drug is insoluble in water and aqueous fluids at the dosage required for administration and when attempts to solubilize the drug through cosolvents, surfactants, and other solubilizing agents compromise the stability or safety and taste properties of a dosage form. The taste of a bitter or unpleasant drug can often be improved by the selection of an insoluble form of the active drug moiety. The large surface area of the dispersed drug, in a suspension dosage form, ensures a high degree of availability for absorption. Unlike tablets or capsules, the dissolution of drug in suspension and subsequent in vivo absorption commence upon dilution in gastrointestinal fluids.

Sweetening agents are often a major constituent of oral liquid dosage forms. Sucrose, the most commonly used sweetening agent, is soluble in aqueous media, sweet, chemically stable in the pH range of 4.0 to 8.0, and is available in highly purified form at a reasonable cost (1). Sucrose has been used as a sweetener to increase the palatability in foods and pharmaceuticals. However, the use of sucrose has been associated with a number of health problems and a reduction of sucrose intake has been advocated in the literature, since consumption of sucrose is often correlated with obesity, diabetes mellitus, and dental caries (4).

In recent years, one of the most active areas of food research and development has been in the area of alternative sweeteners to sucrose. These sweeteners are expected to provide sweetness with fewer or no calories and they are used to: expand food and beverage choice for those who need or want to control caloric, carbohydrate, or sucrose intake; assist in weight control and reduction; aid the management of diabetes; assist the control of dental caries; and enhance the usability of pharmaceuticals and toiletries (5).

Although many alternative sweeteners have been developed, only a few are currently marketed in North America, Europe, and Japan. Sanyude (6) has reviewed alternative sweeteners including: those which have been in use for a long time such as saccharin and cyclamates; alternative sweeteners which have recently been approved for use such as Acesulfame-K^{R}, thaumatin, stevioside, dihydrochalcones; the polyalcohol sweeteners such as xylitol, sorbitol, and mannitol; and aspartame (APM).

Many alternative sweeteners have have limited usefulness. Problems associated with saccharin include a bitter aftertaste and carcinogenicity in laboratory animals (7). The U.S. Food and Drug Administration (FDA) proposed a ban on saccharin in 1977 on the basis that it was carcinogenic (8). Cyclamates were banned in the USA in 1970 because they were also shown to increase the incidence of urinary bladder cancer in laboratory animals (9). Thaumatin and dihydrochalcones have unusual taste profiles and lingering aftertaste. Excessive consumption of polyalcohol sweeteners can cause a laxative effect. Stevioside has not been approved because long term toxicity studies have not been completed.

APM is currently used as an alternative sweetener for sucrose in food and beverages in Canada, the United States, and many other countries. It is about 200 times sweeter than sucrose, has no bitter or metallic aftertaste, is essentially calorie-free, and has been recognized as safe for human consumption (6). The use of APM has increased since it was first marketed in 1974 (10). It is currently used widely in the food industry (11) and it will most probably become more widely used in the pharmaceutical industry in the future.

There is little published information regarding, the use of APM in pharmaceuticals; however, APM has been used in a formulation of Metamucil^R, Questran Light^R, several chewable multivitamin tablets, Neo Citran^R, and chewable Calsan^R tablets, which are marketed in the USA and Canada. APM generally has not been used in wet pharmaceutical systems because under certain conditions of moisture, temperature, and pH, APM hydrolyzes to aspartylphenylalanine and cyclizes to a diketopiperazine, 5-benzyl-3,6-dioxo-2-piperazineacetic acid (DKP) (11,12). Homler and Neirynck have shown that aqueous solutions of APM are unstable at low pHs (pH < 3.0) and at high pHs (pH > 6.0). In the pH range between 3.0 and 5.3 APM solutions are relatively stable; at 22°C the half-life of APM at pH 4.5 is 243 days (13).

1.1 HYPOTHESIS:

In solution, APM decomposes via a series of reactions that include ester and peptide hydrolysis and cyclization to diketopiperazine (11,12,14). The hydrolytic, or solvolytic, decomposition of liquid pharmaceuticals is not a new problem. In many instances, the usual remedies of inhibiting, or avoiding, solvolysis include: judicious maintenance of pH, careful selection of a buffer system and buffer concentration, complexation, use of a less soluble form of the drug, use of surfactants, modification of the chemical structure, and partial or total replacement of water with organic solvents (15-23). For example, the stability of many drugs in solution including barbiturates, diazepam, and aspirin have been significantly increased by the partial or total replacement of water by propylene glycol, ethanol, polyethylene glycol or glycerol (24-26). These non-aqueous organic solvents often reduce hydrolysis by lowering the dielectric constant of the vehicle (26-30) The stability of penicillin in aqueous systems was improved by using the less soluble form, the procaine derivative, and by formulating it with citrate, dextrose , sorbitol or gluconate (15). Also the stability of benzocaine and procaine have been increased by complexation (16).

There are various physical and chemical strategies that can be used to improve the stability of pharmaceuticals in liquid systems (15-23). Thus it was hypothesized that by careful selection of formulation parameters such as vehicle, pH, and certain excipients (i.e., those which minimize the degradation of APM) the stability of APM in liquid pharmaceutical dosage forms could be increased. With an improved shelf-life in wet pharmaceutical systems (3 years or more), APM could be used extensively, as a sweetener and flavor enhancer, in oral liquid dosage forms. With this approach in mind, studies were conducted to determine factors which are important in formulations of APM in liquid systems. The information obtained in these experiments was then used to attempt to optimize the stability of APM in liquid pharmaceutical dosage forms which used APM as the main sweetener.

1.2 AIM:

The purpose of this thesis was to investigate the potential of using APM as a sweetener in liquid pharmaceutical dosage forms. In this investigation the objectives to be achieved included the following:

1. To characterize factors that affect the stability of APM in liquid systems.
2. To optimize the stability of APM in liquid pharmaceutical systems.
3. To formulate and develop liquid pharmaceutical dosage forms containing APM which satisfy the requirements of pharmaceutical elegance with respect to taste, appearance, and rheology.

2. BACKGROUND

2.1 HISTORICAL ASPECTS

The sweet taste of APM was discovered in December 1965 by a G.D. Searle and Company chemist, James Schlatter, during the synthesis of a gastrin tetrapeptide to be used for ulcer therapy (31,32). The APM intermediate spilled on Schlatter's hand. When he licked his fingers to pick up a piece of weighing paper, he tasted the sugarlike taste of the dipeptide ester. Upon the discovery of APM, Searle examined about 200 analogs of APM but finally decided to commercialize the original discovery (33). The reasons for choosing the original compound for commercialization were as follows: none of the analogs could be manufactured any more economically than APM; the potency was satisfactory, and the taste quality was excellent; the components, L-aspartic acid, L-phenylalanine and methanol, are dietary components which the body metabolizes normally; and Searle speculated that APM would have an excellent chance of surviving the most severe and searching toxicity testing (33).

2.1.1 History in the United States

In 1968-69, G.D. Searle and Company contacted the FDA to discuss what toxicological testing should be conducted on APM. Data were submitted to the FDA in 1972-73 and on July 26, 1974, APM was first approved as a sweetener and flavor enhancer for tabletop use and as an ingredient in certain dry food applications (10). Immediately following this approval, formal objections to the regulation were filed. These objections were based primarily on safety concerns, so in December 1975, the FDA stayed the regulatory approval of APM until questions concerning the conduct of certain animal tests, supporting the approval of APM, could be resolved. The animal studies in question were audited and authenticated by the FDA and by an independent, expert organization, the Universities Associated for Research and Education in Pathology. Following the authentication, a series of scientific issues were presented and discussed before a panel of three scientific experts (Public Board of Inquiry), by representatives of the FDA, G.D. Searle and Company, and objectors to the regulation of APM. The decision of the Public Board of Inquiry was published on October 1, 1980 (33) and the FDA Commissioner's final decision, published on July 24, 1981, concluded APM had been shown to be safe (34). On July 8, 1983 the FDA amended the food additive regulation for APM to permit its use in carbonated beverages and carbonated beverage syrups (35). Recently the FDA approved the use of APM in additional food categories such as frozen stick novelties, ready-to-eat gelatin and ready-to-drink, aseptically packaged fruit drinks (36).

2.1.2 Worldwide Status

APM has been approved for food and beverage use in many countries around the globe. Countries with the broadest use approval are the United States, Canada, U.K., Japan, South Africa and Switzerland (37).

2.2 MANUFACTURING

The manufacturing technology for the production and purification of APM is described by patents (38-40). The manufacture is complicated by the fact that only the α form of L,L,dipeptide ester is the sweet isomer. The D,L, L,D, and the D,D,dipeptide are not sweet and also the β-dipeptide is reported to be bitter (41). The general basic process of synthesis is one of preparing both L-amino acids, and then using them to prepare L-aspartic acid anhydride and the methyl ester of L-phenylalanine which are coupled to produce α-APM.

The following method was used to synthesize α-APM (42). A solution of 90 g of methyl L-phenylalaninate hydrochloride in 450 mL of water was neutralized with 24 g of sodium carbonate, and the resulting free ester was extracted with two 350 mL portions of ethylene dichloride. To the organic solution there was then added 9 g of acetic acid and 8 mL of methanol. Into this solution 15.2 g of L-aspartic acid anhydride was stirred at -20°C. After stirring for 0.5 h at this temperature, 350 mL of water at about 75°C and an aqueous solution of sodium carbonate (5.7 g in 300 mL) were added successively to the reaction mixture. The excess methyl L-phenylalaninate was removed by two extractions with 150 mL portions of ethylene dichloride. Then the aqueous layer was adjusted to pH 4.8 with dilute hydrochloric acid. This solution was found to contain 60% α-APM and 20% ß-APM. Separation of α-APM from ß-APM was achieved by concentrating this solution in a vacuum to about 100 mL and then adding 36% hydrochloric acid to the concentrate. This solution was kept in a refrigerator overnight. Crystals of α-APM.HCl were formed and were collected by filtration. These crystals were dissolved in 200 mL of water and this solution was adjusted to pH 4.8 with a 5% solution of sodium carbonate in water. This solution was kept in the refrigerator overnight. Crystals of α-APM were collected by filtration. The yield of this synthesis procedure was 43% and the α-APM was further purified by ion-exchange chromatography (42).

2.3 PHYSICAL AND CHEMICAL PROPERTIES

α-APM is N-α-L-aspartyl-L-phenylalanine-1-methyl ester and its structure is shown in Fig 1.1. It is an off-white crystalline powder with a molecular weight of 294.3. APM is almost odorless, but sometimes has a slight acetic acid odor. On themogravimetric analysis APM loses weight at 196°C, representing the loss of methyl ester and conversion to DKP, and again at 315°C, representing the decomposition of the DKP. The dissociation constants (pk_a's) of APM at 25°C are $pk_{a1} = 3.1$ and $pk_{a2} = 7.9$. The isoelectric point is 5.2 and, APM has

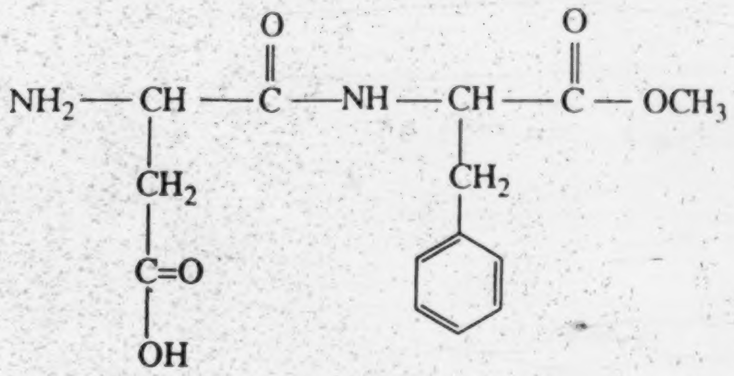


Figure 1.1 Chemical structure of aspartame

a specific rotation in 15N formic acid of +15.91 (37).

2.3.1 Solubility

The solubility of APM in water is dependent on pH and temperature. The solubility increases as temperature increases. APM exhibits minimum solubility (1% solution) in water at its isoelectric point (pH 5.2) and a maximum solubility (10% solution) at pH 2.2. The solubility of APM can be increased in mixed solvents; in water or acetic acid alone a 1% solution is possible, but in 70% acetic acid in water a 30% solution of AMP can be achieved. At pH values below the isoelectric point, the rate and degree of APM solubility can be improved by first dissolving an acid in the system or by dissolving both the acid and APM at the same time. APM exhibits less solubility in organic solvents and oils than in water (4).

2.3.2 Taste

2.3.2.1 Physiology of Taste

The sense organs for taste, the taste buds, are ovoid bodies measuring 50 to 70 µm (43). Each taste bud is made up of 5 to 18 hair cells, the gustatory receptors and supporting cells. The receptor cells each have a number of hairs projecting into the taste pore, the opening at the epithelial surface of the taste bud. Each taste bud is innervated by about 50 nerve fibers. In humans, the taste buds are located in the mucosa of the epiglottis, palate, and pharynx and in the walls of the fungiform and vallate papillae of the tongue. The papillae are rounded structures near the tip of the tongue; the vallate papillae are prominent structures arranged in a V on the back of the tongue. The sensory nerve fibers from the taste buds on the anterior two-thirds of the tongue travel in the chorda tympani of the facial nerve. The fibers from the posterior third of the tongue reach the brain stem via the glossopharngeal nerve. The fibers from areas other than the tongue reach the brain via the vagus nerve. Impulses from these three nerves are ultimately relayed to the taste projection area in the cerebral cortex at the foot of the postcentral gyrus (43).

In humans there are four basic tastes: sweet, sour, bitter, and salt (43). Sweet substances are tasted at the tip of the tongue, sour along the edges, bitter on the back and salt on the dorsum anteriorly. Sour and bitter substances are also tasted on the palate along with some sensitivity to sweet and salt. All four modalities can be sensed on the pharynx and epiglottis. Acids taste sour. The hydrogen ion, rather than the associated anion, stimulates the receptors. For a given acid, sourness is generally proportionate to the hydrogen ion concentration, but organic acids are often more sour for a given hydrogen ion concentration than mineral acids. Salty taste is produced by the anions of inorganic salts. The halogen are particularly effective, but some organic compounds also taste salty. Alkaloids taste bitter. Examples are quinine, morphine, nicotine, caffeine and others. Some inorganic salts of magnesium, ammonium and calcium also taste bitter. The bitter taste is due to the cations. Most sweet substances are organic compounds. These substances have diverse structural features, for examples monosaccharides, disaccharide, amino acids, peptides, proteins, artificial sweeteners, glycerol, chloroform, some alcohols and ketones (43).

2.3.2.2 Molecular Theory of Sweet Taste

Examination of the molecular structures of a large number of sweeteners by Shallenberger and Acree (44) has led to the observation that sweeteners have a common feature - the AH, B system. The AH, B system consist of two electronegative atoms, A and B separated by a distance of 2.5 to 4 Å. The H is a hydrogen atom attached to A by a covalent bond. The A and B are usually either oxygen or nitrogen, but can be carbon, chlorine, or a center of unsaturation. According to the hypothesis, there must be a complemetary AH, B system on the receptor site (an AH - B site) such as a protein peptide bond or glutamine or asparagine amino group. Keir (45), Shallenberger, and Lindley (46) have proposed in addition to the AH, B system, a third lipophilic group, X, to explain the different sweetness intensities of various compounds (47).

It is considered mostly likely by many workers (48,49), that the sweet taste of APM is elicited by the trifunctional unit AH - B - X. Shinoda et al., reported in greater detail the contributions of these trifunctional units for sweetness as well as bitterness (50). Nosho et al., used the AH - B - X system to design, synthesize and test "inverted-aspartame-type sweeteners" (51). These researchers were able to synthesize compounds which were about 50 times sweeter than sucrose and potentially more stable in aqueous solutions than APM. They also concluded from their results that phenyl was sufficient for the lipophilic group, X, and that the optimal distance between AH - B and X was 6 or 7 carbon atoms. The molecular configuration of the sweet compound is also an important consideration. It is believed that the APM produces the strong sweet taste because it has a branched structure at the phenylalanine moiety so that the flexibility of the X functional group (phenyl group) is limited (51).

2.3.2.3 Taste Quality and Intensity

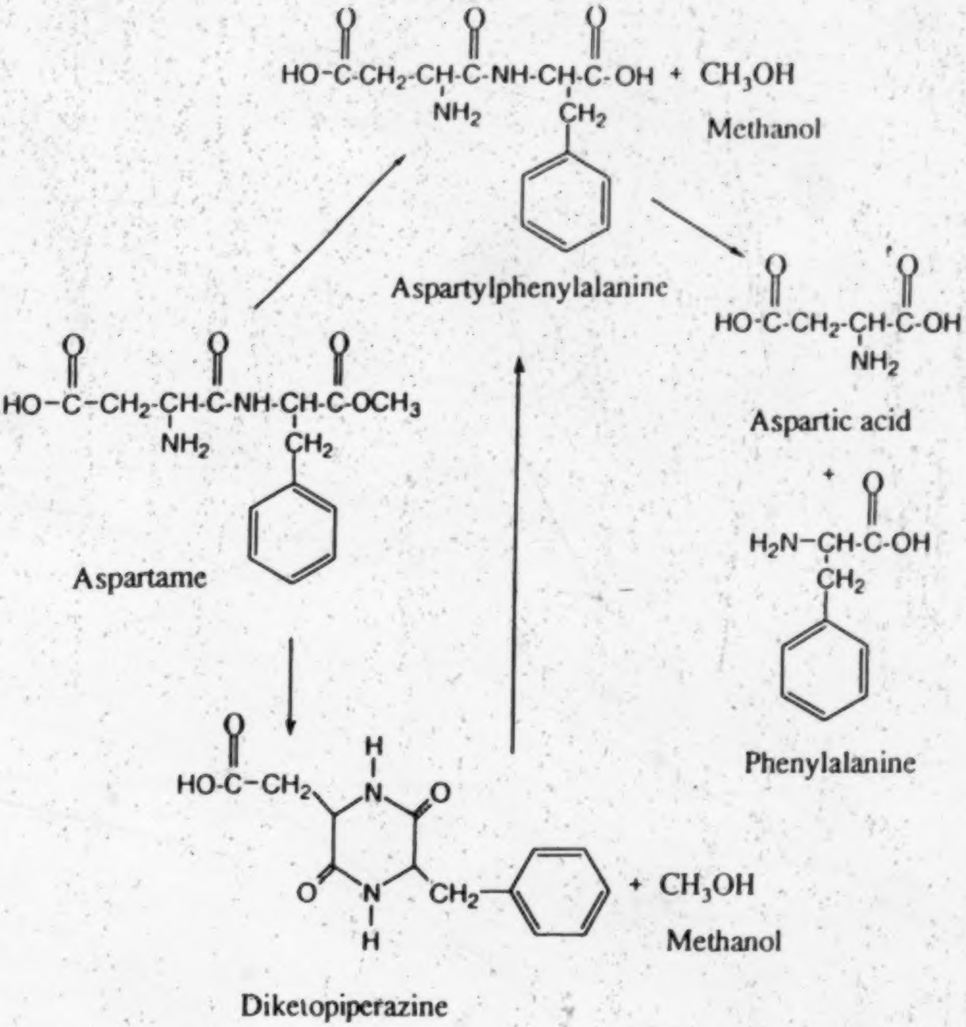
APM has a clean, sweet taste, free of bitter or metallic aftertaste. The sugar-like taste of APM is well documented (52-63). APM's sweetness is inversely related to the concentration of sucrose. At 3% sucrose, APM is 215 times the sweetness of sucrose, but it has only 133 times the sweetness of sucrose at 10% sucrose concentration (64). APM's time versus sweetness intensity profiles in aqueous solutions are very similar to those of sucrose but sweetness of APM is slightly persistent. Any lingering sweetness from APM can be modified by using less sweetener, lactose, or certain salts such as sodium bicarbonate, aluminium potassium sulphate and gluconate salts (65-68). The sweetness intensity of APM can vary depending on the system and also as a result of minor formulation changes such as those involving flavor, solid content or viscosity. APM is also a flavor enhancer. It can influence or potentiate flavors and has been reported to extend some food and beverage flavors, particulary acid fruit flavors. This flavor extension is best with flavors naturally derived rather than with artificial flavors (67).

2.3.3 Stability

Dry APM is stable (4). It is affected only by extremely high temperatures. Samples of APM packaged in standard food-service paper packets have been kept in normal warehouse storage for over 3 years and still met a 95% α-APM specification (4). APM is a dry powder even when it has up to 8% moisture. In dry foods, the stability of APM is not a concern unless it is used in combination with hygroscopic ingredients. The stability of APM is satisfactory for tablets and for table-top sweeteners which are mechanical blends with lactose, maltodextrin, or dextrose. Decomposition under dry conditions occurs primarily by loss of methanol to form the dipeptide and by the simultaneous loss of methanol and water to form the DKP. These changes are very slow (4). In liquid media, APM decomposes principally by two reactions (Scheme I) (37). The ester bond is either hydrolysed to form aspartylphenylalanine and methanol or simultaneously water and methanol are eliminated by the cyclization of APM to form DKP. Water may react with the DKP to form the dipeptide which ultimately can undergo peptide hydrolysis to its individual amino acids, aspartic acid and phenylalanine. The decomposition appears to follow first order kinetics and pH-rate profiles have shown that APM is stable in the pH range of 3.0 to 5.0 (37).

2.3.4 Shelf Life

In the pharmaceutical field, the time required for 10% of the drug to degrade is an important value to know, since it represents a reasonable limit of degradation of active ingredients (69). The expressions for $t_{10\%}$ are given in Table 1.1 (69). The FDA defines the stability of a pharmaceutical product as the capability of a particular formulation, in a specific container/closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications (70). The stability of a drug can also be defined as the time from the date of manufacture and packaging of the formulation until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not


Aspartylphenylalanine
Methanol
Aspartame
Aspartic acid
Phenylalanine
Diketopiperazine
Methanol

SCHEME 1

Table 1.1[a]

Expressions of Half Lives and Shelf Lives of the Zero-, First-, and Second-Order Reactions

	Zero-order	First-order	Second-order
$t_{½}$	$\frac{c_o}{2k}$	$\frac{0.693}{k}$	$\frac{1}{c_o k}$
$t_{10\%}$	$\frac{c_o}{10k}$	$\frac{0.105}{k}$	$\frac{0.11}{c_o k}$

[a]From reference (69)

changed appreciably or deleteriously (70). Although there are exceptions, 90% of the labeled potency is generally recognized as the minimum acceptable potency level. Shelf life of a product is then defined as the time in which the preparation will remain stable when stored under recommended conditions (70).

Knowledge of the product's shelf life is very important for a number of reasons. The stability of the active component is a major criterion in determining the shelf life for two reasons (70). First, there may be chemical degradation of the active ingredient, leading to a substantial lowering of the quantity of the therapeutic agent in the dosage form. Many drugs for example, digoxin and theophylline have narrow therapeutic indices and they may need to be carefully titrated in individual patients so that serum levels are neither too high as to be potentially toxic nor too low as to be ineffective. In these cases, it is of paramount importance that drug dosage form can reproducibly deliver the same amount of drug (69). Second, although chemical degradation of the active drug may not be extensive, toxic product may be formed in the decomposition process. Dearborn (71) described a number of examples in which the products of degradation are significantly more toxic than the orginal therapeutic agent. Thus the conversion of tetracycline to epianhydrotetracycline, arsphenamine to mapharsen, and p-aminosalicylic acid to m-aminophenol in dosage forms give rise to potentially toxic agents which, when ingested, can cause undesirable effects. Physical stability of the dosage form is equally important for three primary reasons (70). One, a pharmaceutical product must appear fresh, elegant, and professional, so long as it remains on the shelf. Any change in physical appearance such as color fading, mottling of tables, creaming of emulsion, caking of suspension, or haziness can cause patients or consumers to lose confidence in the product. Two, since some products are dispensed in multiple-dose containers uniformity of dose content of the active ingredient over time must be assured. A cloudy solution or a broken emulsion can lead to a nonuniform dosage pattern. Three, the active ingredient must be available to the patient throughout the expected shelf life of the preparation. A breakdown in the physical system can lead to nonavailability of the drug to the patient (70).

It is worthwhile, in kinetic studies, to determine the half life of the chemical or drug. The half life, $t_{1/2}$, is the time required for the drug amount to decrease to 50% of its original value, that is, 50% degradation. The general expressions of $t_{1/2}$ are given in Table 1.1. It is also important to note that the $t_{1/2}$ and $t_{10\%}$ for zero- and second-order reactions are dependent on concentration, c_0, whereas for first-order reactions, $t_{1/2}$ and $t_{10\%}$, are concentration independent.

2.4 TOXICITY ASSESSMENT

Because several concerns have been raised about the safety of APM, the toxicity and metabolism of APM have been studied in many experiments. There are excellent review articles on APM, with special emphasis on its potential toxicity (72-75).

APM is composed of substances normally found in the diet and in the body, namely L-aspartic acid, L-phenylalanine and methanol. After oral absorption, APM is rapidly and extensively metabolised in such a way that aspartate, phenylalanine and methanol are released to the portal blood (74). The objections to the use of APM as a food ingredient have been based on concerns about the potential toxicity of each of these three components of APM (74). Very large doses APM or its component parts (aspartate, phenylalanine, methanol) may produce deleterious effects in sensitive animal species (74).

There are many published estimates of APM intake. The Market Research Corporation of America and Stegink et al., have calculated that 34 mg/kg body weight represents the 99th percentile of the projected daily ingestion of APM (76,77). The FDA's current acceptable daily intake (ADI) of APM is 50 mg/kg body weight. The Joint Expert Committee on Food Additives estimated the level causing no effect in the rat to be 4 g/kg body weight/day, and proposed an ADI (safety factor 100) of 40 mg/kg body weight/day for humans (75). The critical question is whether APM is potentially harmful at normal use and potential abuse levels of the product (75).

2.4.1 The Methanol Moiety

Methanol is released during decomposition and metabolism of APM. Tephyl and McMartin (78) have reviewed metabolism and toxicity of methanol. Ingestion of large quantities of methanol elevates blood methanol and formate concentrations, which leads to a variety of adverse effects, such as metabolic acidosis, blindness and even death. Stegink (79) and Pardrige (80) assessed the issue of potential adverse effects caused by methanol when APM is consumed (75). Both authors concluded that the amount of methanol released does not pose any hazard to humans when the sweetener is consumed at normal use levels (50 mg/kg body weight). Evidence to support this conclusion is that the use of APM in soft drinks provides methanol amounts not exceeding the methanol amounts normally present in fruit juices. For example a typical sweetened soft drink would have 555 mg/L of APM, which is equivalent to 60 mg/L methanol. This is considerably lower than the average methanol content of fruit juice (140 mg/L) (74). A bolus intake of APM at 40 mg/kg body weight is equivalent to 4.4 mg/kg body weight of methanol. This ingestion level is well below the methanol exposure levels required to achieve toxic reactions in humans; such levels are equal to or greater than 1 g/kg body weight (80).

2.4.2 The Aspartate Moiety

The dicarboxylic amino acids aspartate and glutamate have been shown to induce neuronal necrosis in the arcuate nucleus of the hypothalamus in the brain of neonatal mice (81). These amino acids are additive with regard to this effect, but, they only exert the neurotoxic effects when very high doses are administered.

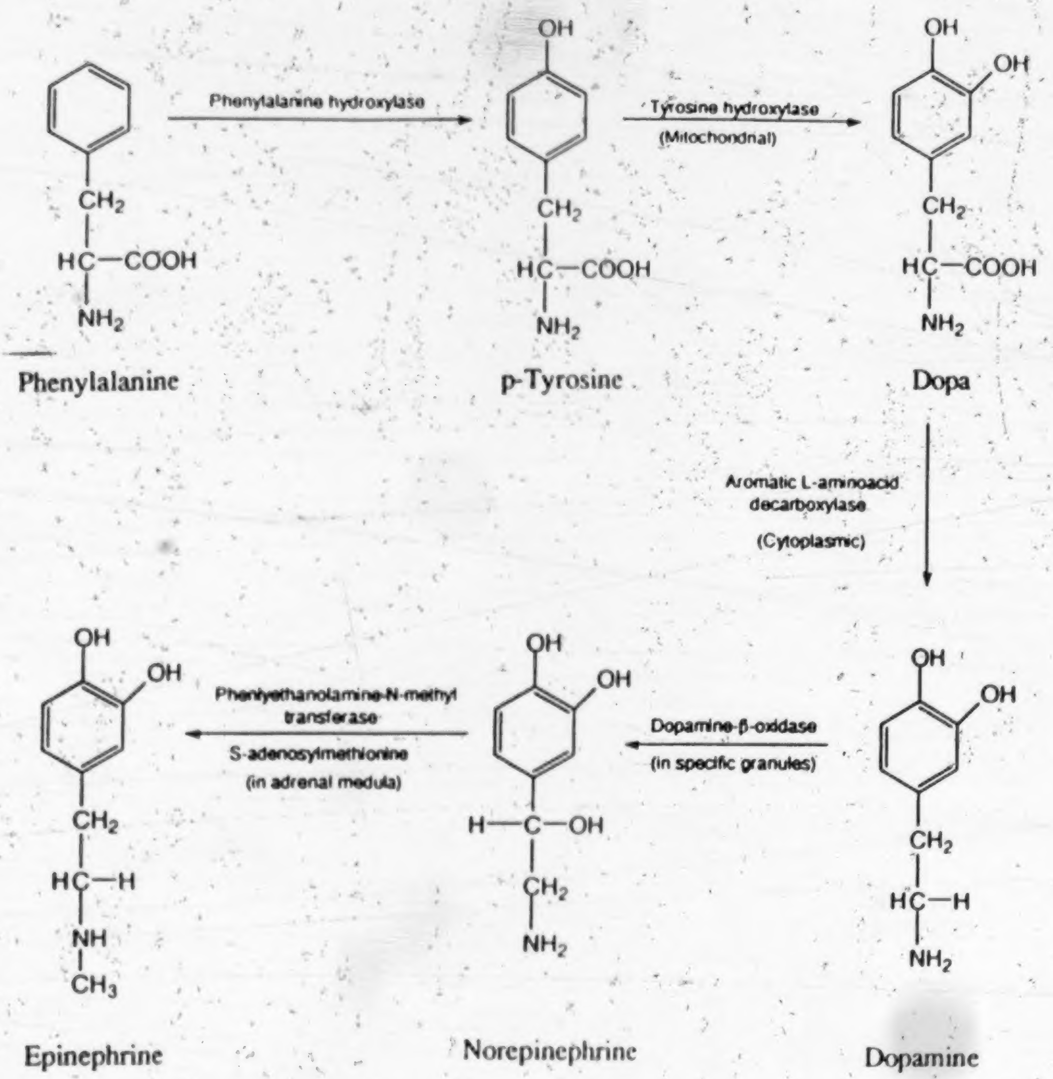
Although considerable intra-species variation in aspartate and glutamate metabolism has been demonstrated in mice, rats, monkeys and humans (82-94), neonatal rodents given large doses of APM or aspartate developed hypothalamic lesions. There is disagreement over the ability of these dicarboxylic amino acids to produce similar neuronal lesions in neonatal primates. Olney et al. (85) reported glutamate-induced lesions in neonatal monkeys, but four other research groups were unable to reproduce these findings (86-91). In one study, the administration of APM at 2 g/kg body weight to infant nonhuman primates, with or without glutamate (1 g/kg body weight), did not produce neuronal necrosis, despite grossly elevated plasma aspartate and glutamate levels (92). In neonatal mice, lesions were consistently found in all studies with both dicarboxylic amino acids. The lowest effective doses were 500 mg/kg body weight and 650 mg/kg body weight for glutamate and aspartate respectively (93). Applebaum et al. (81) concluded that in neonatal mice a sum plasma concentration of glutamate and aspartate of 600-1000 μmoles/L must be reached before neuronal necrosis in the hypothalamus occurs. In neonatal rats the lowest effective doses for monosodium glutamate and potassium aspartate were 2000 mg/kg body weight and 440 mg/kg body weight respectively (92). The available data do not provide convincing evidence that the characteristic hypothalamic lesions observed in rodents also occur in primates.

In clinical human studies reviewed by Stegink (76), normal subjects were administered APM at 34 mg/kg body weight or aspartate at 13 mg/kg body weight in randomised crossover design. No significant differences in plasma concentrations from baseline values were noted. This indicates rapid metabolism of the aspartate portion of APM. Even when APM was given at 100, 150 or 200 mg/kg body weight, the mean high plasma aspartate levels were well below normal postprandial levels observed in orally fed human infants and adults, and were far below levels producing lesions in mice. Similar studies were performed in human infants and, again, the plasma aspartate levels were not elevated significantly. These results show that even at bolus doses of APM exceeding the ADI limit, in both adults and infants, plasma concentrations remained well below the toxic threshold for neonatal mice (600-1000 μmoles/L.) These results also demonstrate that there is rapid metabolism and clearance of the aspartate portion of APM in humans (75). Baker reported the absence of a significant effect on amino acid concentrations in breast milk of lactating women after single oral administration of 50 mg/kg body weight (96). He found no placental transfer of glutamate and aspartate in rhesus monkeys when the plasma concentrations of these amino acids were 2000 μmoles/L and 1000 μmoles/L respectively. Chronic ingestion studies were performed, and the results also indicated that there was no significant accumulation of aspartate. Therefore, it can be concluded that the aspartate portion of APM at normal use or abuse levels of APM dose not contribute to toxicity (75).

2.4.3 The Phenylalanine Moiety

The metabolism of APM yields, on a weight basis, approximately 50% phenylalanine, 40% aspartic acid and 10% methanol (97). Phenylalanine is a neutral amino acid which is essential for humans. The initial step in the metabolism of phenylalanine is hydroxylation to tyrosine. Tyrosine is a precursor of the neurotransmitters dopamine and norepinephrine (Scheme II) (98). So phenylalanine via tyrosine may affect the brain levels of dopamine and norepinephrine and thus influence brain function (99,100).

The entry of large neutral amino acids (LNAAs) from the blood into the brain is by a stereospecific and saturable transport system (75). Through this system phenylalanine, tyrosine, leucine, isoleucine, valine, tryptophan, histidine and methionine are transported into the brain. These LNAAs compete for available transfer sites for entry into the brain.


Phenylalanine hydroxylase
Tyrosine hydroxylase
(Mitochondrial)
Aromatic L-aminoacid decarboxylase
(Cytoplasmic)
Dopamine-β-oxidase
(in specific granules)
Phenylethanolamine-N-methyl transferase
S-adenosylmethionine
(in adrenal medula)
Phenylalanine
p-Tyrosine
Dopa
Dopamine
Norepinephrine
Epinephrine

SCHEME II

Imbalances in the plasma amino acid concentrations may perturb the brain uptake of these amino acids. For example, when the plasma phenylalanine concentration is elevated, without a corresponding increase in other LNAAs, the uptake in the brain of phenylalanine increases at the expense of other LNAAs. An increase in phenylalanine or tyrosine uptake might reduce the uptake of tryptophan, which is a precursor of serotonin, and increased phenylalanine plasma concentrations could influence the biosynthesis of serotonin in the brain, and thus affect brain function (80,101). It has been reported however, that a better predictor of phenylalanine uptake into the brain is the ratio of plasma phenylalanine to the plasma level of all other LNAAs (102,103).

Phenylketonuria (PKU) is an genetic defect of phenylalanine metabolism which is caused by a hereditary absence or defect in the ability to hydroxylate phenylalanine to tyrosine. There are three forms of PKU, each corresponding to the dysfunction of one of the three coenzymes participating in the phenylalanine hydroxylase system (104). The classical form of PKU is caused by the total or near-total absence of hepatic phenylalanine hydroxylase activity. Plasma phenylalanine concentrations are markedly elevated (≥ 1200 μmoles/L) and if the disease in neonates remains untreated, it may result in delayed development, electroencephalitic abnormalities, mental retardation, and, in some cases, seizures (99). In most hospitals newborn infants are tested for this disease, which can be diagnosed by blood phenylalanine concentration of 1200 μmoles/L or greater. When it is detected early in life, and the patient is placed on a phenylalanine-restricted diet, normal development is possible. This genetic disease is due to the homozygous state of a single autosomal recessive gene which has an incidence of 1 in 60 individuals (72).

From the above observations it was determined that high plasma phenylalanine levels reduce the uptake into the brain of other LNAAs (102) and thus cause the observed harmful effects to the developing brain. In accordance with this, Caballero and Wurtman argued that the balance between the relative intake of phenylalanine and other LNAAs is the main factor limiting phenylalanine's access to the brain (105). In normal food proteins the phenylalanine content compared to that of other LNAAs is usually low. Because of this, it is important to determine to what extent consumption of APM might contribute to phenylalanine imbalance manifested in PKU.

Two research approaches have been developed to explore the effects of consumption of APM (75). One line of research focused on the measuring of the plasma phenylalanine concentrations in humans after consumption of APM and comparing these with toxic concentrations found in PKU patients. The other approach was the study of concentrations of phenylalanine, tyrosine, tryptophan and neurotransmitters such as dopamine, norepinephrine and epinephrine in plasma and in brain of experimental animals (101,105,106,107).

In evaluating human plasma phenylalanine concentrations after APM consumption, distinction should be made among normal, PKU heterozygous, and PKU homozygous subjects.

2.4.3.1 Normal Subjects

In adults the fasting plasma phenylalanine concentration is in the range 46-67 μmoles/L, and after a protein-rich meal, levels of 90-120 μmoles/L are reached (104,108). The fasting phenylalanine concentration in fetal and infant plasma is 105 and 77 μmoles/L respectively (104). Many researchers have examined phenylalanine levels after both acute and multiple doses of APM (109,110). Interpolation from the data of Filer and Stegink (110) indicates that after a single bolus oral dose of APM (40 mg/kg body weight) to an adult, the plasma phenylalanine level increases to 130 μmoles/L. These researchers showed by calculation that repeated administration of APM at 34 mg/kg body weight at 8-hour intervals would bring about a steady-state plasma concentration of phenylalanine to 60 μmoles/L. When the same dose was given at time intervals of 4, 3, 2, and 1 hour(s), steady-state plasma phenylalanine concentrations increased to 90, 100, 120, and 180 μmoles/L respectively (110). Stegink and coworkers also examined plasma phenylalanine levels when APM was given as part of a meal. After a protein-rich meal to which APM (23 mg/kg body weight) was added, the peak plasma level was 120 μmoles/L as compared to 97 µmoles/L after the meal alone. However, when 34 mg/kg body weight of APM was added to low-protein meal, the peak plasma phenylalanine level was 145 µmoles/L. From this data it is apparent that the presence of protein (other amino acids) moderates the increase in plasma phenylalanine concentrations. Overall, the results of such studies demonstrated that APM consumption by normal adults at the ADI level entails no risk of toxic levels of phenylalanine in plasma.

Stegink et al. (110) reported a study in which normal infants (1-year-olds) were given a single dose of APM in a beverage. The test doses of 34, 50, and 100 mg/kg body weight produced plasma phenylalanine concentrations of 94, 116, and 233 µmoles/L respectively versus 49 µmoles/L in the controls. Visek (111) reported the results of a 13-week-study with groups of children ranging in age from 2 to 21 years. APM was given as part of a normal diet at dose levels of 30-35 mg/kg body weight/day for children 6 years and younger or 30-77 mg/kg body weight for older children. This incomplete report states that no increases in plasma phenylalanine concentrations were found. These studies provide no evidence that, in normal infants, consumption of APM at ADI levels produces phenylalanine concentrations in the toxic range.

2.4.3.2 PKU Heterozygotes

Persons heterozygous for PKU have a reduced capacity to metabolise phenylalanine because of a reduction in phenylalanine hydroxylase activity (99). In PKU heterozygotes, plasma phenylalanine is usually slightly elevated and clearance of phenylalanine is slower than in normal adults (99). Several studies done in PKU heterozygotes measured plasma phenylalanine concentrations after ingestion of APM (76,79,109,111). A number of single dose studies are reported and the results are in agreement with the results reported by Stegink and coworkers (76,79,109). They indicated that single bolus dose intake at ADI levels by PKU heterozygotes would cause plasma phenylalanine to increase to about 200 µmoles/L. In one multiple dose study after eight doses of 8.5 mg/kg body weight at one-hour intervals, the high mean plasma phenylalanine level was 165 µmoles/L (109). Most studies show that after APM consumption at the ADI level, even when the entire dose is consumed as a single bolus, plasma phenylalanine concentrations will not rise to levels commonly associated with adverse effects.

2.4.3.3 PKU Homozygotes

When PKU homozygotes are not placed on a phenylalanine-restricted diet, plasma concentrations of phenylalanine rise to 1200-3600 µmoles/L and mental retardation occurs (75). For children on phenylalanine-restricted diet, a plasma concentration of 480 µmoles/L is regarded as the upper limit permitted in Western Europe (75). Consumption of APM at ADI levels by young PKU homozygotes contributes significantly to the phenylalanine content of the diet. Gutler and Lou (113) have found marked increases in plasma phenylalanine levels in three nine-year-old homozygous patients after a single dose of 34 mg/kg body weight of APM. In older PKU patients, the consumption of APM at ADI levels significantly elevates the plasma phenylalanine. Consequently it is prudent that all PKU patients on phenylalanine-restricted diets avoid consumption of APM. Those PKU patients on phenylalanine-liberalised diets should be made aware of the phenylalanine content of APM.

It is recommended that during pregnancy in PKU homozygotes the maternal plasma phenylalanine concentrations be controlled so that they do not exceed 380 μmoles/L. If maternal plasma phenylalanine is not controlled there is a risk of phenylalaninemia in fetal blood which might cause mental retardation. Phenylalanine levels in fetal blood are usually twice those in maternal blood.

2.4.4 Consumer Complaints

In the USA, consumer complaints about APM have been made to several institutions and to the manufacturer. These adverse reactions reports have been evaluated by the Center for the Disease control on behalf of the FDA in 1984 (114) and by the Center for Food Safety and Applied Nutrition of the FDA in 1986 (115). The initial analysis of 517 cases from 1984 was updated and extended to 2800 cases in 1986. The reported symptoms were grouped according to the nature and severity of the reaction and by the frequency and consistence of the association with ingestion of APM (114-116). Additional analysis focused on demographics, specific product or lot association, and consistency of dose and time and response relationships with an individual and within case-defined reporting groups. Complainants reporting severe reactions were interviewed and their medical records were obtained. The reported complaints included the following categories: neurological seizures, 100 cases; headache, 832 cases; dizziness, 383 cases; changes in mood quality, 310 cases; gastrointestinal, 387 cases; allergies, 239 cases; and others such as menstrual irregularities, sleep problems and visual disturbances. Roberts (117) reviewed 505 APM-related complaints and identified pregnant women, lactating mothers, young children and older persons with memory impairment as high-risk groups. The main conclusion drawn from these complaints was that no symptom complex has been identified which supports the contention that no general public health hazard is associated with APM consumption (115). Most of the frequently reported symptoms were mild and were common in the general population. In most cases a causal relationship between APM consumption and the complaints was considered to be questionable.

2.4.5 Concluding Remarks on Safety

There is no significant risk for aspartate-induced neurotoxic effect in brain when APM is consumed at levels within the ADI level. Most of the experimental evidence indicates that this conclusion holds for adults, infants, and fetuses (75).

Phenylalanine at plasma levels of 1200 µmoles/L or greater is toxic to the human brain. At lower concentrations no firm conclusions can be made because controversy exists about the question of whether the deleterious effects on brain function, caused by hyperphenylalaninemia, follow linear or threshold patterns. Definitive studies in this area are lacking, but it is clear that there are no observational data supporting the view that adverse effects occur at moderately elevated plasma phenylalanine concentrations (200-400 µmoles/L). In normal adults evidence shows that after APM ingestion at ADI levels, plasma phenylalanine concentrations are practically always within the normal postprandial range; elevation to plasma concentrations commonly associated with adverse effects has not been observed. This statement holds for PKU heterozygous adults and probably heterozygous infants and pregnant women. However, ingestion of APM at ADI levels would significantly increase the phenylalanine intake of PKU homozygotes, thus deranging the phenylalanine restriction maintained by these patients. PKU homozygotes should avoid APM.

Analyses of complaints by the FDA have not yielded a specific constellation of symptoms clearly associated with APM consumption that would suggest a widespread public health hazard related to APM use. More clinical studies, which could provide evidence to assist in the interpretation of these adverse reaction reports of APM, are underway in the USA (75).

DKP and ß-APM are decomposition products or impurities of APM that have also been subjects of many toxicological studies (118,119). They have both been found to have no pharmacologic activity and to be noncarcinogenic and nonmutagenic (118,119). Available experimental evidence shows that when these compounds are ingested in the amounts associated with the normal use or potential abuse levels of APM, they are not toxic to humans (118,119)

2.5 SELECTION OF DRUGS

Acetaminophen and diazepam were the drugs selected for this study. Acetaminophen was used in the solution dosage form while diazepam was used in the suspension dosage form. The primary reasons choosing these drugs were the following:

(a) Both drugs have bitter taste so they need sweetening agents in their oral liquid dosage forms.

(b) They are stable in liquid dosage forms and the mechanisms of degradation of both drugs have been characterized satisfactorily.

(c) They are among the most commonly used drugs in patients such as pediatric and geriatric patients, who often have difficulty in swallowing solid dosage forms.

(d) Both drugs are stable in the pH range where APM exhibits reasonable stability.

2.5.1 Acetaminophen Stability

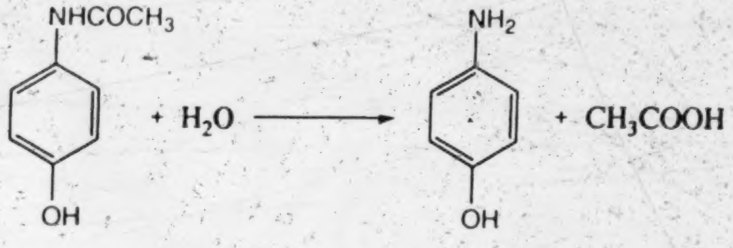
Acetaminophen is very stable in aqueous solution. Its pH-rate profile reveals specific acid and specific base catalysis with maximum stability in the pH range of 5.0 to 7.0 (120). The major route of acetaminophen degradation is its hydrolysis to p-aminophenol and acetic acid (Scheme III). Koshy and Lach studied the stability of acetaminophen in aqueous solution. They found out that spontaneous hydrolysis was negligible and that important reactions were equations 1 and 2.

$$\text{HO-C}_6\text{H}_4\text{-NHCOCH}_3 + \text{H}^+ \xrightarrow[\text{H}_2\text{O}]{k_H} \text{HO-C}_6\text{H}_4\text{-N}^+\text{H}_3 + \text{CH}_3\text{COOH} \qquad (1)$$

$$\text{HO-C}_6\text{H}_4\text{-NHCOCH}_3 + \text{OH}^- \xrightarrow[\text{H}_2\text{O}]{k_{OH}} \text{HO-C}_6\text{H}_4\text{-NH}_2 + \text{CH}_3\text{COO}^- \qquad (2)$$

This rate equation can be written as equation 3.

$$\text{rate} = k_H[\text{H}^+][\text{APAP}] + k_{OH}[\text{OH}^-][\text{APAP}] \qquad (3)$$



Acetaminophen p-Aminophenol

SCHEME III

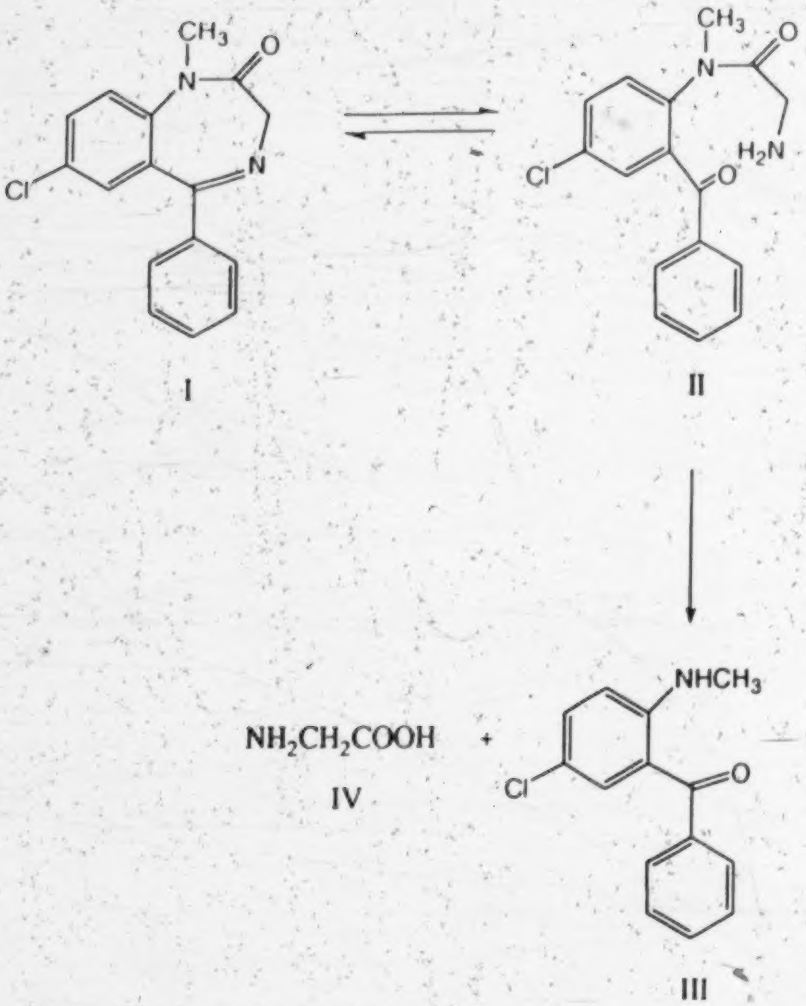
where k_H and k_{OH} are specific acid and specific base rate constants, respectively, and APAP represents acetaminophen. At constant pH, the reaction is pseudo first-order with the apparent first-order rate constant k' being related to k_H and k_{OH} by equation 4.

$$k' = k_H[H^+] + k_{OH}[OH^-] \qquad (4)$$

The pH-rate profile shows that maximum stability occurs at pH of 6.0 and that at this pH and at 25°C the half-life of acetaminophen in aqueous solution is calculated to be 21.8 years. The rate of degradation of acetaminophen was also shown to be insensitive to ionic strength (120,121).

2.5.2 Diazepam Stability

Diazepam (I) is one of the more stable of the substituted 1,4-benzodiazepines (122,123). It undergoes hydrolysis in aqueous solutions, with a benzophenone as the major decomposition product. The degradation proceeds via an open-ring intermediate (II), leading to the formation of 2-methylamino-5-chlorobenzophenone (III) and glycine (IV) (see Scheme IV). This reaction is pH-dependent. At pH values less than the pK_a, the intermediate is not subject to recyclization so the degradation kinetics are biphasic (equation 5). At pH values greater than pK_a, recyclization of the intermediate is facile and the degradation kinetics are monophasic in



SCHEME IV

character (equation 6) (122,123). The pk_a of diazepam is 3.3 at 20°C.

$$\text{I} \longrightarrow \text{II} \longrightarrow \text{III} \qquad (5)$$

$$\text{I} \rightleftarrows \text{II} \longrightarrow \text{III} \qquad (6)$$

The observed first-order rate constant reported for diazepam hydrolysis at 80°C over a pH range of 4.0 to 7.0 was < 1.7 x 10^{-7} s^{-1} and maximum stability in aqueous solution was at approximately pH 5.0 (123). Carstensen *et al.* studied the hydrolysis of diazepam in mixed solvent systems and their results show that diazepam is more stable in mixed solvents than in aqueous solutions (123).

3. EXPERIMENTAL SECTION

3.1 MATERIALS

The drugs and reagents used in these studies were: acetaminophen[1], diazepam[2], aspartame[1], aspartylphenylalanine[1], L-phenylalanine[1], diketopiperazine[3] acetonitrile[4] (HPLC grade) and Avicel^R RC-591[5].

All other chemicals and solvents used were of reagent grade. The water used in the preparation of solutions and HPLC mobile phases was de-ionized and glass-distilled.

3.2 METHODS

3.2.1 Stability of APM in Buffered Aqueous Solutions.

The objective of this kinetic study was to characterize factors that affect the stability of APM in buffered aqueous solutions. Factors studied were pH, buffer species, buffer concentration, and ionic strength. These preformulation studies are a necessary first-step in developing stable oral liquid dosage forms in which APM is used as the sweetener.

---

[1] Sigma Chemical Co., St. Louis, MO, USA.
[2] F.Hoffman-La Roche and Co. Ltd., Basle, Switzerland.
[3] U.S.P.C. Inc., Rockville MD, USA.
[4] Mallincdrot, Inc. Paris, Kentucky, USA.
[5] FMC Corproation, Philadelphia, PE, USA.

3.2.1.1 Kinetic method. The study was performed on 5 mg/mL APM solution in four different buffer systems (acetic acid buffer, citric acid buffer, malic acid buffer, and tartaric acid buffer). Sodium chloride was used to adjust the ionic strength. The final volume of each sample was 25 mL. Sample containers were 50 mL amber glass bottles which were tightly closed with black plastic caps, containing vinyl liners. The samples were put in waterbaths set at 40°, 50° and 60°C, (±1°C). Samples were allowed 30 minutes to equilibrate, then the initial aliquots (1 mL) were withdrawn. Samples were taken from the waterbath and cooled in an icebath for about 2 minutes before the withdrawal of the aliquots. Other aliquots were withdrawn at predetermined intervals until the concentration of APM remaining in the solution was one-half or less of the initial concentration. The level of residual APM, in each sample, was determined at least six times. The rate constant of degradation was calculated from the slope of the plot of the natural logarithm of the residual APM versus time.

3.2.1.2 Analytical method. A reverse phase HPLC method was used to follow the disappearance of APM in the samples with time. This HPLC assay is a modification of the method of Verzella and Mangia (124). A Waters HPLC system[6] equipped with a multiple wavelength UV detector (Model 450)[6] and a model 3390A recorder integrator[7] was used. The stationary phase was a μBondapak Phenyl[R6] (column dimensions were 3.9 mm, internal diameter, and 30 cm, length; particle size, 10 μm). The mobile phase was 5% (v/v) acetonitrile in 0.01 M potassium hydrogen phosphate, pH 2.5. The UV detector, set at 215 nm, was used with guaifenesin (1 mg/mL) as the internal standard. The flow rate was 1.5 mL/min. Chart speed was 1 cm/min, and the temperature was ambient.

Calibration curves were constructed from the results of the injections of five solutions. These solutions contained 0, 0.25, 0.5, 0.75, and 1.0 mL of APM standard solution (2 mg/mL) and 0.5 mL of the internal standard; and their final volume was made up to 2 mL with 0.005 M malic acid buffer. The calibration curves were then constructed by plotting the ratio of peak area of APM to guaifenesin against the known amount of APM in the solution. The concentrations of the samples were calculated from these calibration curves. The calibration procedure was done whenever the determination of

---

[6] Waters Associates Inc., Milford, MA, USA.
[7] Hewlett Packard Co., Palo Alto, California, USA.

APM was carried out. Samples were prepared in exactly the same way as the solutions used in the calibration curves. One-half-milliter of the sample was put in a test tube, 0.5 mL of internal standard and 1 mL of malic acid buffer were added. The test tube was vortexed for 1 minute to mix the contents and 10 μL was injected onto the HPLC column.

3.2.1.3 Experimental design. The application of factorial experimental designs to preformulation studies has been reported for various pharmaceutical systems (125-128). Factorial experimental designs were used in this experiment because these designs have the following advantages (129-130):

(a) they are the designs of choice when many factors are studied concurrently;

(b) in the absence of interactions, factorial designs have maximum efficiency in estimating the main effects;

(c) if interactions are present, factorial designs are necessary to reveal and to identify them;

(d) since factors are measured over varying levels of other factors, conclusions apply to a wide range of conditions;

(e) maximum use is made of data since main effects and interactions are calculated from all the data; and

(f) factorial designs are orthogonal, that is, estimated effects and interactions are independent of each other.

In this study a 2^3 factorial design was used. For each of the four buffer systems, the effect of the three factors: 1.) pH (A); 2.) ionic strength (B); and 3.) buffer concentration (C); were studied at two levels (low and high), and at three temperatures (40°, 50° and 60°C). The low and high levels of the factors were as follows: pH, 4.3 and 5.3; ionic strength, 0.6 and 0.8; and buffer concentration, 0.1 M and 0.2 M. Table 1 is the calculation matrix for a 2^3 factorial design, with the following combinations of factors A, B, and C at two levels: (1), a, b, ab, c, ac, bc, and abc. In these combinations (1) refers to all factors at low level; (a) refers to the experiment with factor A at the high level and B and C at low levels; (b) refers to the experiment with factor B at the high level and A and C at low levels; and (ab) refers to the experiment with factors A and B at high levels and C at low level, etc.

3.2.1.4 Statistical procedure. The main effects and interactions were calculated from the totals of the individual treatment combinations by means of a table of signs (Table 1) and by the Yates Method (129,130). The experimental error (i.e., error mean square) of each experiment was estimated.

3.2.2 Stability of APM in Mixed Solvent Systems.

This investigation was a study of the effect of solvent composition on the decomposition of APM. In order to examine the influence of solvent composition on the stability of APM in solution, the degradation of APM was carried out in water-methanol, water-ethanol, water-glycerol mixtures with calculated apparent dielectric constant values of 45, 55, and 65. The rate of disappearance of APM was measured by the HPLC assay which was described in the preceding section.

3.2.2.1 Preparation of samples

Three solvent systems, water-ethanol, water-methanol and water-glycerol, and three storage temperatures, 40°, 50° and 60°C, were used in this study. At each storage temperature, mixtures of these solvent systems were prepared with dielectric constant values of 45, 55, and, 65 (24). Equation (7) was used to calculate the fraction of the organic solvent in these mixtures:

$$f_{org} = \frac{D_{mix} - D_{water}}{D_{org} - D_{water}} \qquad (7)$$

where f_{org} is the fraction of the organic solvent; D_{mix} is the dielectric constant of the solvent mixture; D_{water} is the dielectric constant of water; and D_{org} is the dielectric constant of the organic solvent. Because dielectric constants change with temperature, the values used in this study for ethanol, methanol, and glycerol are shown in Table 3.2 (24). At each temperature, three samples were prepared for each solvent system using the solvent mixtures with dielectric constant values of 45, 55, and 65. Each sample was prepared in duplicate so there were in total 18 samples at each of the storage temperatures. The composition of all the samples was the same except for the alcohol or glycol concentration. A 0.05 M malic acid buffer, pH 4.5 (± 0.1) ionic strength 0.26 (adjusted by the addition of sodium chloride) was used. The samples (final volume 25 mL) were put in water baths set at 40°, 50° or 60°C, (±1°C). Samples were allowed 30 minutes to equilibrate then the initial aliquots (1 mL) were withdrawn. Other aliquots were taken at predetermined intervals until the concentration of APM remaining in the solution was one-half of the initial concentration or less. Residual APM, in each sample, was assayed at least six different times in order to determine the degradation rate constant.

3.2.2.2 Experimental Procedure for the pH-Rate Profile

In this experiment only the ethanol-water solvent system was used. The solvents used were water [dielectric constant at 60°C is 66.6 (Table 3.2)]; water-ethanol 24.7% [dielectric constant at 60°C is 55]; and water-ethanol 45.9% [dielectric constant at 60°C is 45 (Table 3.3)]. Duplicate samples of APM, 5 mg/mL, were prepared at pH 2.5, 3.5, 4.5, 5.5, and 6.5.

Table 3.1

Calculation Matrix for a 2^3 Factorial Design.

Factor Combination	Level of Factor in Experimental[a]			Level of the Interactions[b]			
	A	B	C	AB	AC	BC	ABC
(1)	-	-	-	+	+	+	-
a	+	-	-	-	-	+	+
b	-	+	-	-	+	-	+
ab	+	+	-	+	-	-	-
c	-	-	+	+	-	-	+
ac	+	-	+	-	+	-	-
bc	-	+	+	-	-	+	-
abc	+	+	+	+	+	+	+

[a] -, factor at low level; +, factor at high level.
[b] Multiply signs of factors to obtain signs for interaction terms in combination (e.g. AB at (1)=(-)x(-)=(+)).

for each of these three solvents. The apparent pH values of samples were adjusted as described in the preceding section. Ionic strength was also adjusted to 0.26 with sodium chloride. The samples were equilibrated in a waterbath at 60°C for 30 minutes. Initial aliquots (1 mL) were then withdrawn. Other aliquots were taken at predetermined intervals until the concentration of APM remaining in the solution was one-half of the initial concentration or less. Residual APM in each sample was assayed at least six times in order to determine the degradation rate constant. The experiment was repeated.

Table 3.2

Dielectric Constants of Solvents at
Different Temperatures (24).

Solvent	Dielectric Constant at Temperatures		
	40°C	50°C	60°C
Ethanol	22.20	20.87	19.55
Methanol	29.03	27.44	25.97
Glycerol	37.30	35.53	33.82
Water	73.12	69.85	66.62

Table 3.3

Chemical Composition of the Hydro-Organic Mixtures at Dielectric Constant Values of 65, 55, and 45.

Solvent System	Dielectric Constant	Percent of Organic Solvent (v/v) at		
		60°C	50°C	40°C
	65	3.4	9.9	15.9
Water-Ethanol	55	24.7	30.3	35.6
	45	45.9	50.7	55.2
	65	4.0	11.4	18.4
Water-Methanol	55	28.6	35.0	41.1
	45	53.2	58.6	63.8
	65	4.9	14.1	22.7
Water-Glycerol	55	35.4	43.3	50.6
	45	65.9	72.4	78.5

3.2.3 Further Elucidation of APM's Degradation in Aqueous Solution

The degradation of APM in aqueous solutions has been shown to occur primarily by two reactions Scheme I. The ester bond hydrolyses to form the dipeptide and methanol or simultaneously water and methanol are eliminated by the cyclization of APM to form the diketopiperazine. The aim of this experiment was to determine the relative magnitude of these two reactions in the pH range of 3.0 to 5.3.

The starting solutions in this study were APM (5 mg/mL) and aspartlyphenylalanine (0.70 mg/mL). These solutions were made in 0.05 M malic acid buffer. The pH values of the samples were adjusted to 3.0, 4.3 and 5.3. At each of the three pH values four samples of APM and four samples of aspartylphenylalanine were prepared. Samples were stored in waterbaths set at 40°, 50°, and 60°C. So at each of these three temperatures there were twelve samples of APM and twelve samples of aspartylphenylalanine.

3.2.3 1 Analytical method The analytical method used was the same HPLC assay described for APM except that here the decomposition products were also quantitated. Calibration curves were constructed from the results of injections of five solutions. These solutions contained 0, 0.25, 0.5, 0.75, or 1.0 mL of standard solution and 0.5 mL of the internal standard, and the final volume of each solution was made up to 2 mL with 0.005 M malic acid buffer. The standard solutions which were used were APM (2 mg/mL), aspartylphenylalanine (0.75 mg/mL), diketopiperazine (0.5 mg/mL), and L-phenylalanine (0.5 mg/mL). For each standard solution, a calibration curve was constructed by plotting the ratio of peak area of the standard reagent to internal standard against the known concentration of the reagent in the solution. Concentrations of the samples were calculated from these calibration curves. The calibration procedure was done whenever the analyses of the samples were carried out.

When samples of APM were degraded, the disappearance of APM and the formation of aspartylphenylalanine, diketopiperazine, and phenylalanine were determined. When aspartylphenylalanine was the starting material, the disappearance of aspartylphenylalanine and the formation of the diketopiperazine and phenylalanine were determined.

3.2.4 Formulation of Acetaminophen Solution Dosage Form

A number of formulations of acetaminophen solution were formulated and prepared. The objective was to prepare a sucrose-free solution dosage form in which APM was the sweetener and the use of ethanol was avoided because this dosage form was intended for children. Buffer systems used were citric acid buffer, malic acid buffer, and tartaric acid buffer. The formulation shown in Table 3.4 was chosen as the prototype formulation for further evaluation because it had the best taste qualities. However, formulations with malic acid buffer and tartaric acid buffer also had acceptable taste properties.

The preparation of acetaminophen solution dosage form was as follows: acetaminophen (2400 mg) was dissolved in a solution of polyethylene glycol (12 mL), propylene glycol (12 mL), and glycerol (8 mL). APM (500 mg), citric acid monohydrate (1051 mg), and Kool Aid®[8] (50 mg) were dissolved in about 30 mL of water. Methylparaben (150 mg) and propylparaben (15 mg) were dissolved in sorbitol (25 mL) at about 60°C. Then the three solutions were combined. Magnetic stirrers were used to facilitate dissolution and mixing. The apparent pH of the solution dosage form was adjusted to 4.3 with sodium or potassium hydroxide. Water was used to bring the volume of solution to 100 mL.

3.2.5 Compatibility of Ingredients in Acetaminophen Solution Dosage Form

The purpose of this study was to evaluate the compatibility of the formulation ingredients. A PU 8700 Series UV/Visible scanning spectrophotometer[9] was used to determine whether complexes were formed in the acetaminophen dosage form.

---

[8] General Foods Inc., Don Mills, Ont., Canada.

[9] Philips, Pye Unicam Ltd., Cambridge, England.

Table 3.4

Acetaminophen Solution Dosage Form

Ingredient	Quantity
Acetaminophen	2400 mg
Aspartame	500 mg
Methylparaben	150 mg
Propylparaben	15 mg
Kool-Aid (cherry)	50 mg
Citric acid monohydrate[10]	1051 mg
Sorbitol (70%)	25 mL
Polyethylene glycol 400	12 mL
Propylene glycol	12 mL
Glycerol	8 mL
Water, to make	100 mL

[10] The apparent pH of the dosage form was adjusted to 4.3

Solutions of each of the four ingredients, acetaminophen (12.8 μg/mL), APM (2.50 mg/mL), methylparaben (28.8 μg/mL), and propylparaben (20.0 μg/mL) were prepared. Also solutions which contained combinations of any two of these ingredients were made. These solutions were prepared in a solvent system composed of citric acid buffer (pH 4.3) and the solvents used in the acetaminophen dosage form. The solvents were used in the same proportions as those used in the acetaminophen solution dosage form.

The samples were scanned from 200 nm to 800 nm and UV/visible spectra of the formulation ingredients were obtained. For the samples which contained combinations of ingredients, UV/visible difference spectra were also obtained. These samples were stored for two days and the entire procedure was repeated. The spectra were examined to see if there was any evidence of stable complexes formed.

3.2.6 Stability of the Acetaminophen Solution Dosage Form

The purpose of this experiment was to investigate the chemical stability of APM and acetaminophen in the acetaminophen solution dosage form. Augmented factorial experiments were utilized to find conditions which could maximize the stability of APM. The formulation of acetaminophen solution in Table 3.4 was the prototype for the preparation of the formulations used in the study. In these formulations malic acid buffer replaced citric acid buffer and

Kool Aid® (the colorant-flavorant) was omitted. The same variables examined in the aqueous solutions namely pH, buffer concentration, % organic solvent and temperature were also evaluated in this pharmaceutical dosage form.

3.2.6.1 Experimental Design A modified central composite design was employed and is shown in Table 3.5. It consisted of five levels of each of the variables. The design required 25 runs (formulations) and the experiment was replicated. To estimate the effects of the variables on the stability of APM, data were analyzed by the Yates Method and they were also fitted to a first-order regression equation:

$$Y=b_0+b_1x_1+b_2x_2+b_3x_3+b_4x_4+b_{12}x_1x_2+b_{13}x_1x_3+b_{14}x_1x_4+b_{23}x_2x_3+b_{24}x_2x_4+b_{34}x_3x_4+e \qquad (8)$$

where Y = the response, b_0 = the intercept, b_i = the linear effect, b_{ij} = the interaction effect of x_i and x_j, x_1 = pH, x_2 = buffer concentration, x_3 = solvent composition (%organic solvent), and x_4 = temperature (°C). The five levels of the variables were coded as $-a^{11}$, -1, 0, +1, $+b^{11}$, the decoded values of the variable are in the appendix, Table A.1.

---

[11] -a and +b do not have equal magnitude from the center; this means that the axial formulations are not orthogonal to each other and to the factorial formulations.

3.2.6.2 Analytical Method. A reverse phase HPLC method was used to follow the disappearance of acetaminophen in the samples with time. This HPLC assay is a modification of the method of Sena, et al. (131). A Waters HPLC system[6] equipped with a multiple wavelength detector (Model 450)[6] and a model 3390A recorder integrator[7] was used. The stationary phase was a µBondapak Phenyl®[6]. The mobile phase was 0.005 M tetrabutylammonium chloride in distilled water containing 15% (v/v) acetonitrile. The pH of the mobile phase was adjusted to 6.0 with dilute phosphoric acid or potassium hydrogen phosphate. The UV detector, set at 254 nm, was used with benzoic acid (1 mg/mL) as the internal standard. The flow rate was 2.0 mL/min, the chart speed 1 cm/min, and the temperature ambient.

Calibration curves were constructed from the results of the injections of five solutions. These solutions contained 0, 0.25, 0.5, 0.75, and 1.0 mL of acetaminophen standard solution (100 µg/mL) and 0.5 mL of the internal standard; and their final volume was made up to 2 mL with distilled water. The calibration curves were then constructed by plotting the ratio of peak area of acetaminophen to benzoic acid against the known amount of acetaminophen in the solution. The concentrations of the samples were calculated from these calibration curves. The calibration procedure was done for every acetaminophen determination.

One milliliter of the sample was diluted to volume with

Table 3.5

Matrix of a Modified 2^4 Factorial Central Composite Experimental Design

Formulations		Factor Levels in Coded Form			
No.	Type	x_1	x_2	x_3	x_4
1	factorial	-1	-1	-1	-1
2	factorial	+1	-1	-1	-1
3	factorial	-1	+1	-1	-1
4	factorial	+1	+1	-1	-1
5	factorial	-1	-1	+1	-1
6	factorial	+1	-1	+1	-1
7	factorial	-1	+1	+1	-1
8	factorial	+1	+1	+1	-1
9	factorial	-1	-1	-1	+1
10	factorial	+1	-1	-1	+1
11	factorial	-1	+1	-1	+1
12	factorial	+1	+1	-1	+1
13	factorial	-1	-1	+1	+1
14	factorial	+1	-1	+1	+1
15	factorial	-1	+1	+1	+1
16	factorial	+1	+1	+1	+1
17	central	0	0	0	0
18	axial	-a	0	0	0
19	axial	+b	0	0	0
20	axial	0	-a	0	0
21	axial	0	+b	0	0
22	axial	0	0	-a	0
23	axial	0	0	+b	0
24	axial	0	0	0	-a
25	axial	0	0	0	+b

distilled water in a 200 mL volumetric flask. One milliliter of this diluted sample was then pipetted into a test tube, and 0.5 mL of the internal standard and 0.5 mL of distilled water were added. The test tube was vortexed to mix the contents and 20 µL of this solution was injected into the column of the HPLC. APM was assayed as described previously.

3.2.7 Formulation and Evaluation of Diazepam Suspension

The purpose of this study was to develop a diazepam suspension to evaluate its organoleptic properties and to characterize its physical and chemical stability. A number of diazepam suspension formulations were developed and prepared. The formulation listed in Table 3.6 was chosen for further evaluation, among many other formulations tested, because it had the best taste quality .

The diazepam suspension was prepared as follows. A blender was used to disperse 30 g of Avicel® RC-591 in a liter of water for ten minutes at highspeed. APM, malic acid and artificial cherry extract[11] were dissolved in water. The parabens were dissolved in sorbitol 70%. These preparations were mixed in such a way that the final concentration of Avicel® RC-591 was 1.5%. The diazepam powder (particle size ≤ 90 µm)[12] was levigated with glycerol in a glass mortar and then incorporated in the suspension vehicle. The pH of the

---

[11] Brooke Bond Inc., Belleville, Ont. Canada.
[12] Tyler Seive Co.,

Table 3.6

Diazepam Suspension Dosage Form

Ingredient	Quantity
Diazepam	100 mg
Aspartame	500 mg
Methylparaben	180 mg
Propylparaben	20 mg
Avicel® RC-591	1500 mg
Artificial Cherry Extract	1 mL
Malic acid buffer[13]	0.05 M
Sorbitol (70%)	30 mL
Glycerol	-
Water, to make	100 mL

[13] Concentration of the buffer was in moles /liter.

suspension was adjusted to 4.30 with sodium hydroxide or potassium hydroxide.

3.2.7.1 Kinetic Method. Eight 150 mL samples of diazepam suspension were prepared and placed in 200 mL clear bottles. Four bottles were stored at 5°C in a refrigerator and other four bottles were stored at 35°C in a waterbath. These samples were allowed an hour to equilibrate before the initial tests were conducted. Before chemical analysis, all samples were visually inspected for signs of color change, microbial growth, ease of redispersion and pouring. At each test interval, the pH of each sample was determined with an Accumet® model 320 expanded scale research pH meter[14].

3.2.7.2 Analytical Method. The chemical stability of both diazepam and APM were evaluated by stability-indicating high performance liquid chromatographic assays. The HPLC assay for APM was the same as described previously. At specified times during the 60-day storage period, 3 mL aliquots of the suspension were removed from each bottle at each temperature and placed in 15 mL centrifuge tubes. A 2 mL quantity of methanol and 1 mL of water were added to each sample; the tube was tightly closed and vortexed for 1 minute. The sample was then centrifuged at 2000 r.p.m. for 15 minutes. The supernatant was used for the diazepam and APM HPLC assays.

---

[14] Fisher Scientific Co., Fair Lawn, N.J., USA.

A reverse phase HPLC method was used to follow the disappearance of diazepam in the samples with time. This assay is a modification of an HPLC assay used by Emery and Kowtko (132). A Waters HPLC system[6] equipped with a multiple wavelength UV detector (Model 450)[6] set at 254 nm and a model 3390A recorder integrator[7] were used. The stationary phase was a μBondapak C_{18} column[R6] (column dimensions: 3.9 mm internal diameter, and 30 cm length; particle size 10 μm). Fifteen microliter injections were made. The flow rate was 1.5 mL/min., the chart speed was 1 cm/min., and the temperature was ambient. The mobile phase were various mixtures of acetonitrile and water. Quantitative results were obtained with a 35:65 acetonitrile-water mobile phase. The internal standard stock solution was prepared by pipetting 30 mL of benzene into a 100-mL volumetric flask and bringing to volume with methanol.

Calibration curves were constructed from the results of injections of five solutions. These solutions contained 0, 0.25, 0.5, 0.75, and 1.0 mL of diazepam standard solution (0.5 mg/mL) and 0.5 mL of the internal standard. The final volume of each of these solutions was made up to 2 mL with a 50% (v/v) solution of ethanol in water. The calibration curves were then constructed by plotting the ratio of peak area of diazepam to benzene against the known concentration of diazepam in the solution. The concentrations of the samples were calculated from these calibration curves. The calibration procedure was done whenever the determination of diazepam was carried out.

Samples were prepared in the same way as the solutions used in the calibration curves. One milliter of the supernatant of the centrifuged sample was put in a test tube, 0.5 mL of internal standard and 0.5 mL of ethanol-water solution were added. The test tube was vortexed for 1 minute to mix the contents and 15 µL was injected onto the HPLC column.

3.2.7.3 Rheological Evaluation of Diazepam Suspension

The purpose of this experiment was to determine the flow properties of the diazepam suspension dosage form. A Haake Rotovisco viscometer[16] with an M 150 measuring head and an MV II P cup and rotor was used. The temperature of the samples was maintained at 20.2°C (±0.3°C) with a Lauda[17] RC6 recirculating constant temperature bath. The fluid used in the bath was Silicone 200 fluid[18].

After instrument setup, 55 mL of diazepam suspension was put in a "profile" cup of the viscometer and the rotor was lowered into the sample. This sample was left undisturbed in the viscometer for 24 hours, then the rheogram was plotted the following day. The viscometer was programmed such that shear rate was increased from 0 to 44 s^{-1} in 5 minutes, then was held constant at 44 s^{-1} for 2 minutes and it was decreased from 44 s^{-1} to the initial value of 0 in 5 minutes. The suspension was shaken by hand and another rheogram was generated immediately after shaking. The shear rate was varied as described above.

---

[16] Haake Buchler Instruments Inc., Saddle Brook, N.J., USA.
[17] Brinkmann Instrument Co., Westbury, New York, USA.
[18] Dow Corning Corporation, Midland, Michigan, USA.

4. RESULTS AND DISCUSSION

4.1 STABILITY OF ASPARTAME IN BUFFERED AQUEOUS SOLUTIONS.

Chromatogram in Figure 4.1 is a result of analyzing a typical sample containing APM and some of its degradation products. It shows that the assay was stability-indicating. The degradation products of APM did not interfere with the determination of APM. The ratios of peak areas of APM to internal standard for the calibration curve are shown in Table 4.1. Figure 4.2 was constructed using data from Table 4.1, run number 3. One additional point, peak-area-ratio 0.0 and APM concentration 0.0, was added. Calibration was done using new standards prepared on the day when APM analysis was performed.

The kinetic data in this study were examined by the following equation:

$$\ln c_t = \ln c_o - k \qquad (9)$$

where c_o is the initial concentration of aspartame; c_t is the final concentration of aspartame at time t; t is time in hours; and k is the rate constant. The plot of natural logarithm of concentration of aspartame remaining in the sample versus time was linear with negative slope. Figure 4.3 illustrates a typical plot of the

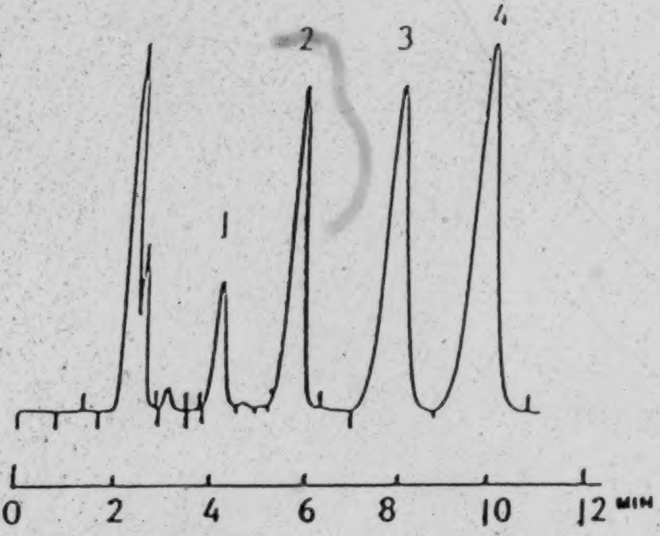


Figure 4.1. HPLC Chromatogram of A Sample with Aspartame (3) and its Degradation Products, Aspartylphenylalanine (1) and 5-Benzyl-3,6-Dioxo-2-Piperazineacetic Acid (2), and the Internal Standard, Guaifenesin (4).

Table 4.1

Validation of Aspartame Analysis by Peak-Area Ratio Measurement

Run No.	Aspartame Concentration (mg/mL)			
	0.29	0.58	0.88	1.16
1	0.835	1.669	2.432	3.217
2	0.830	1.662	2.424	3.220
3	0.827	1.638	2.420	3.194
4	0.828	1.645	2.420	3.215
Mean	0.830	1.660	2.427	3.212
S.D.	0.004	0.010	0.011	0.012
%RSD	4.82	6.02	4.53	3.74

Linear regression: A(peak-area-ratio) = mC(mg/mL) + b
where m = 2.719 and b = 0.054;
correlation coefficient $R^2 = 0.999$

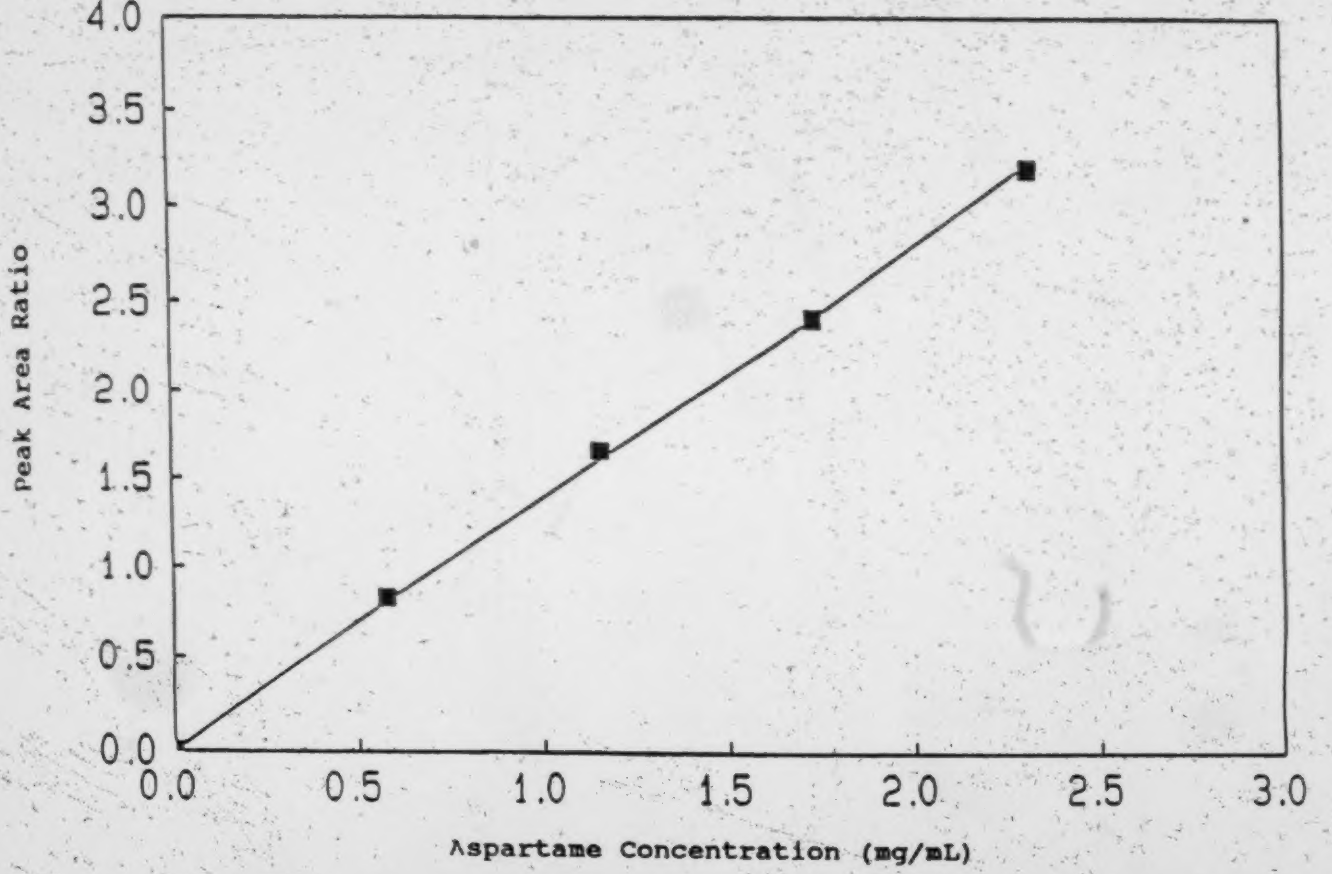


Figure 4.2. Standard Calibration Curve of Aspartame in 0.01 M Malic Acid Buffer at pH 4.0.

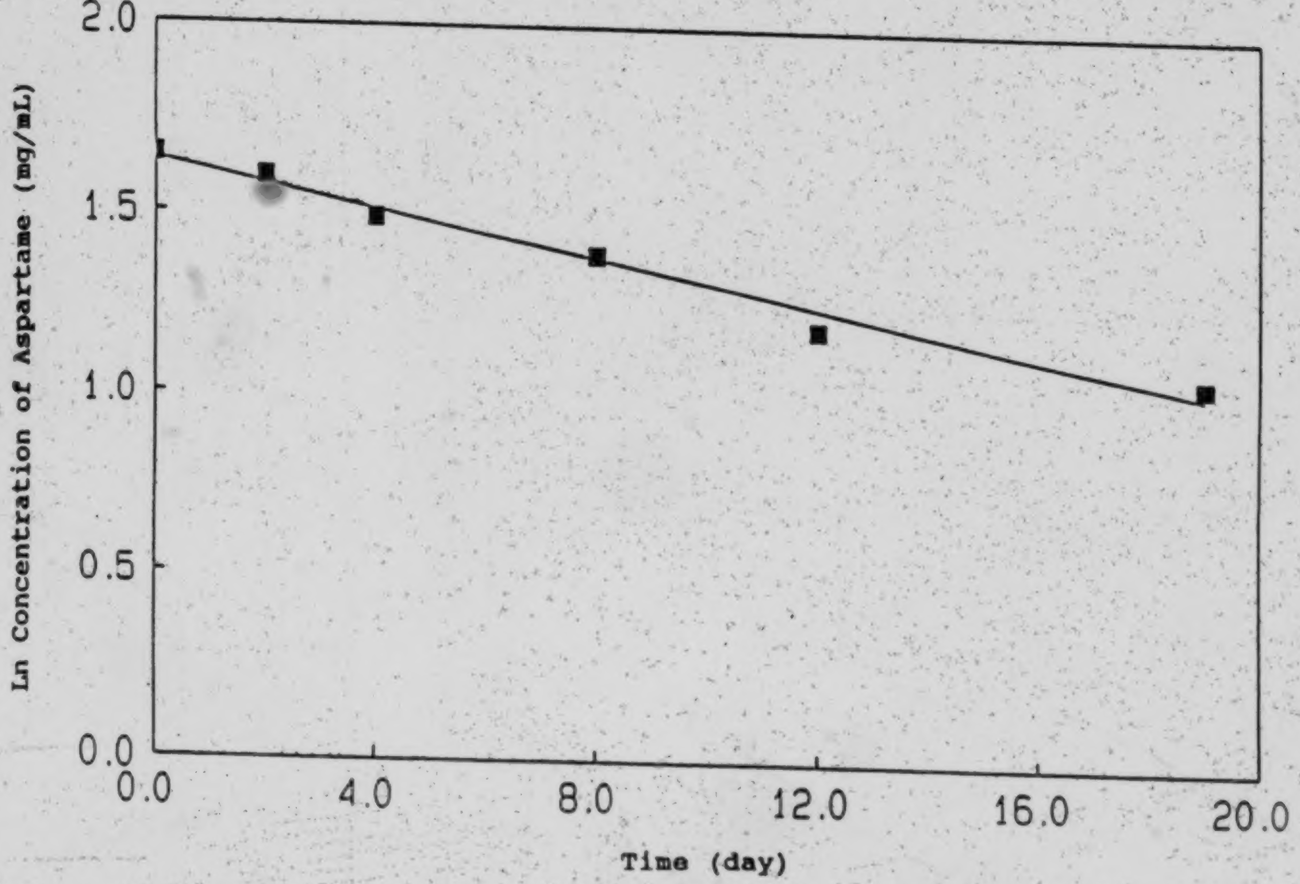


Figure 4.3. Pseudo First Order Degradation of Aspartame in 0.1 M Malic Acid Buffer at pH 3.0 and 50°C.

degradation of APM in aqueous buffer solutions. The linear correlation coefficient for Figure 4.3 was -0.99 and all formulations showed similar linear correlation coefficients.

Table 4.2 to 4.4 summarize the data and statistical analysis of the results of the effect of pH (A), ionic strength (B), and buffer concentration (C) on the degradation rate of aspartame stored at 40°C, 50°C, and 60°C in aqueous acetic acid buffer solutions. The rest of the results of APM degradation in aqueous buffer solutions are presented in Table A.2 to A.13, listed in the Appendix. Diagrams in Figures 4.4 to 4.6 provide visualization of the main effects of factors A, B, and C and their interactions. The lines labeled "Main Effect" are averages of all the data at low and at high levels of the factors in each diagram.

The main effect of increasing pH was to increase the rate constant of degradation of APM. As can be seen in Figure 4.4a, the main effect of increasing pH from 4.3 to 5.3 was to increase the rate constant of APM degradation from 6.0×10^{-3} h^{-1} to 18×10^{-3} h^{-1}. This pH effect is also shown in Table 4.2 as the calculated effect of pH, a, which has a value of 11.96×10^{-3} h^{-1} and it is highly significant (1 d.f., F = 121.79). All diagrams in Fig. 4.3 show that as pH was increased from 4.3 to 5.3, the average effect of pH (lines labelled "Main Effect") was to increase the degradation rate constant. The effect of pH was significant ($p < 0.05$) for all four buffer

Table 4.2

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Acetic Acid Buffer Solutions at 60°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	5.12	4.35	-	-	-	-
a	15.12	14.16	1	11.96	286.08	121.79*
b	4.65	4.78	1	-0.18	0.06	0.03
ab	14.05	13.19	1	-0.56	0.63	0.27
c	7.61	7.86	1	5.97	71.16	30.30*
ac	25.61	20.14	1	2.56	13.11	5.58*
bc	9.67	7.38	1	0.35	0.24	0.10
abc	22.28	22.57	1	-0.06	0.01	0.00
Error			8		2.349	

[a] Significance level based on 1 d.f. *$p<0.05$.

Table 4.3

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Acetic Acid Buffer Solutions at 50°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr)		d.f.	Calculated Effect	Mean Square	F[a]
	Run 1	Run 2				
(1)	1.51	1.86	-	-	-	-
a	4.51	4.79	1	4.055	32.89	1365.28*
b	1.65	1.75	1	0.29	0.16	6.75*
ab	4.91	4.79	1	0.19	0.72	30.38*
c	2.32	2.56	1	1.84	6.73	279.58*
ac	7.15	7.26	1	1.00	2.00	83.03*
bc	2.47	2.62	1	0.18	0.06	2.69
abc	7.95	7.96	1	0.10	0.02	0.75
Error			8		0.024	

[a] Significance level based on 1 d.f. *$p<0.05$.

Table 4.4

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Acetic Acid Buffer Solutions at 40°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	0.46	0.44	-	-	-	-
a	1.53	1.82	1	15.60	485.16	515.44*
b	0.49	0.52	1	0.53	0.55	0.59
ab	1.56	1.74	1	0.43	0.36	0.38
c	0.83	0.80	1	7.78	120.90	128.45*
ac	2.85	2.79	1	3.73	27.75	29.48*
bc	1.01	0.88	1	0.38	0.28	0.30
abc	2.83	2.84	1	0.03	0.00	0.00
Error			8		0.941	

[a] Significance level based on 1 d.f. *$p<0.05$.

systems and at all the three temperatures studied (see calculated effect of a, in Tables 4.2 to 4.4 and Tables A.2 to A.13, listed in the appendix).

The effect of ionic strength on the degradation of APM was not significant. Diagrams in Figure 4.5 show that when ionic strength was increased from 0.6 to 0.8, all the lines had either small negative slopes or are horizontal. This indicates that the rate constant was either slightly decreased or remained the same when ionic strength was increased in this range. The effect of ionic strength was not significant for the four buffer systems and at the three temperatures studied (see calculated effect of b, in Tables 4.2 to 4.4). The effect of ionic strength did not interact significantly with either the effect of pH or the effect of buffer-ion concentration. This is indicated by the fact that all the lines in the diagrams in Fig. 4.5, are paralell within experimental error.

As shown in Tables 4.2 to 4.4, the calculated effect of buffer concentration, c, was to increase the rate constant and this effect is significant ($p < 0.05$). In Figure 4.6a, at pH (A) equal to 5.3, and at ionic strength (B) equal to 0.8, an increase in buffer-ion concentration from 0.1 M to 0.2 M resulted in an increase in the rate constant from 13.6×10^{-3} h^{-1} to 22.3×10^{-3} h^{-1}. All the lines labeled "Main Effect" in Fig. 4.6 have positive slopes. The effect of buffer-ion concentration was significant ($p < 0.05$) for the four buffer

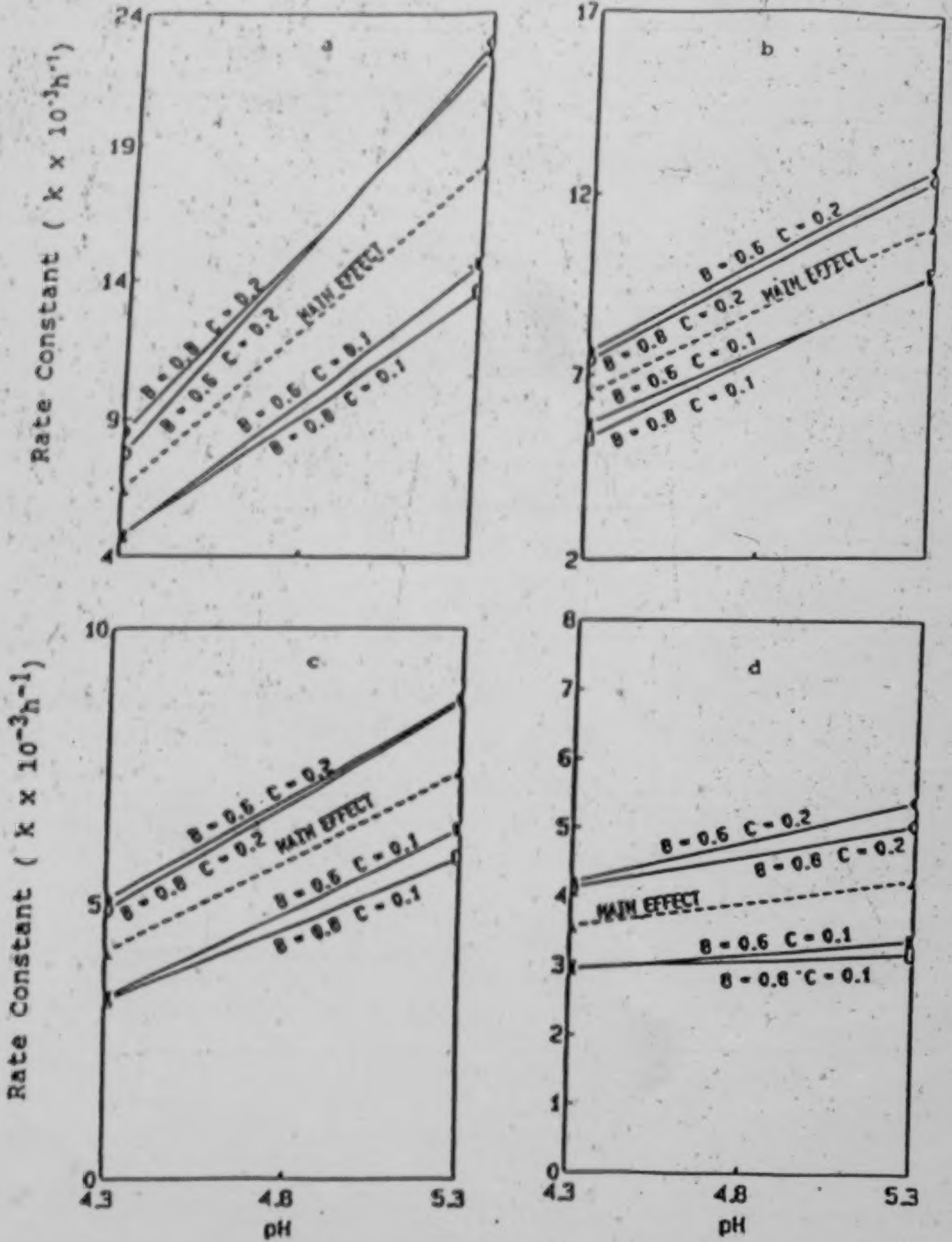


Figure 4.4. The Effect of pH, Ionic Strength (B), and Buffer-Ion Concentration (C) on the Degradation Rate of Aspartame in (a) Acetic Acid Buffer, (b) Citric Acid Buffer, (c) Malic Acid Buffer and, Tartaric Acid Buffer at 60°C.

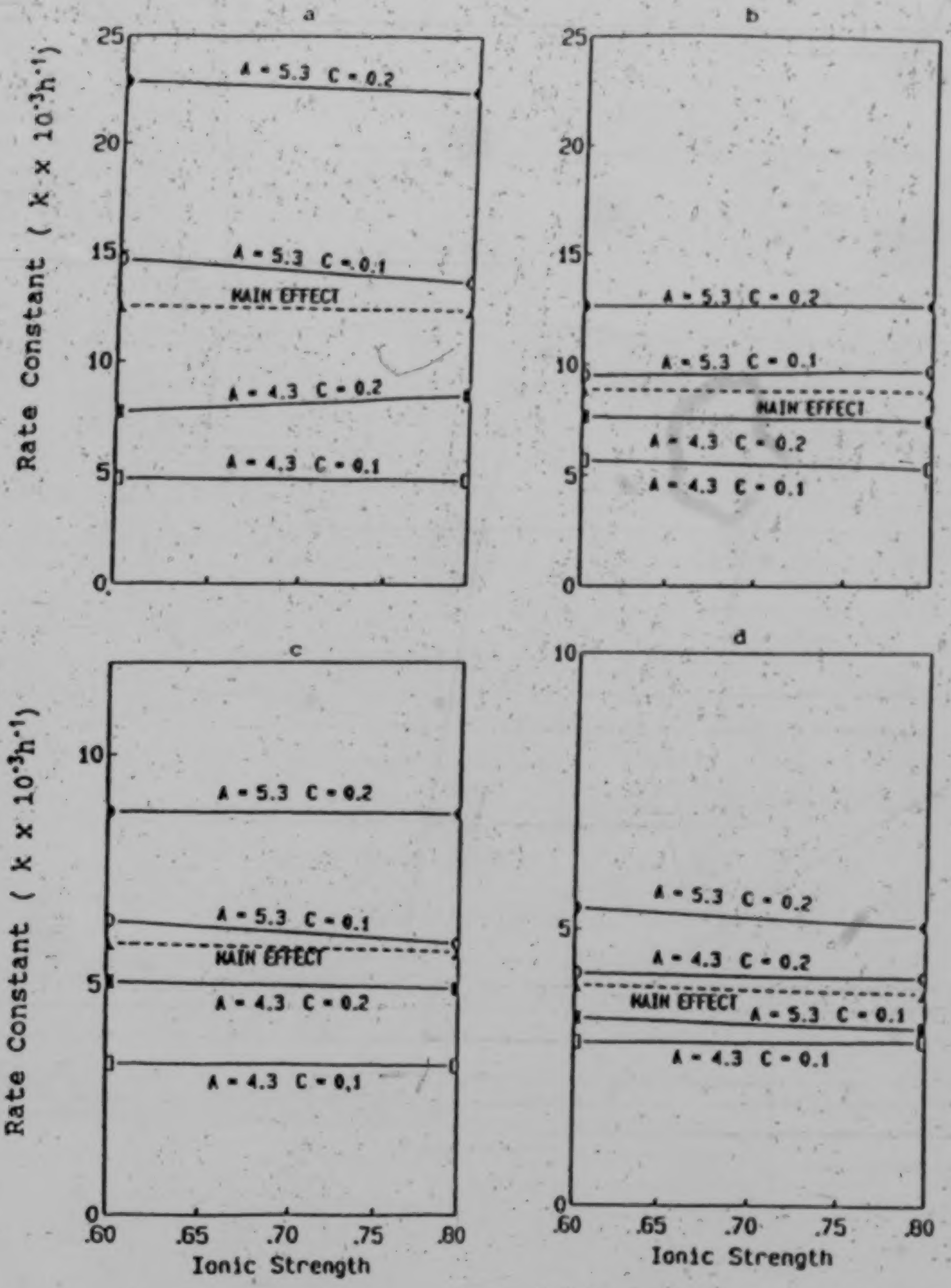


Figure 4.5. The Effect of Ionic Strength, pH (A), and Buffer-Ion Concentration (C) on the Degradation Rate of Aspartame in (a) Acetic Acid Buffer, (b) Citric Acid Buffer, (c) Malic Acid Buffer and, Tartaric Acid Buffer at 60°C.

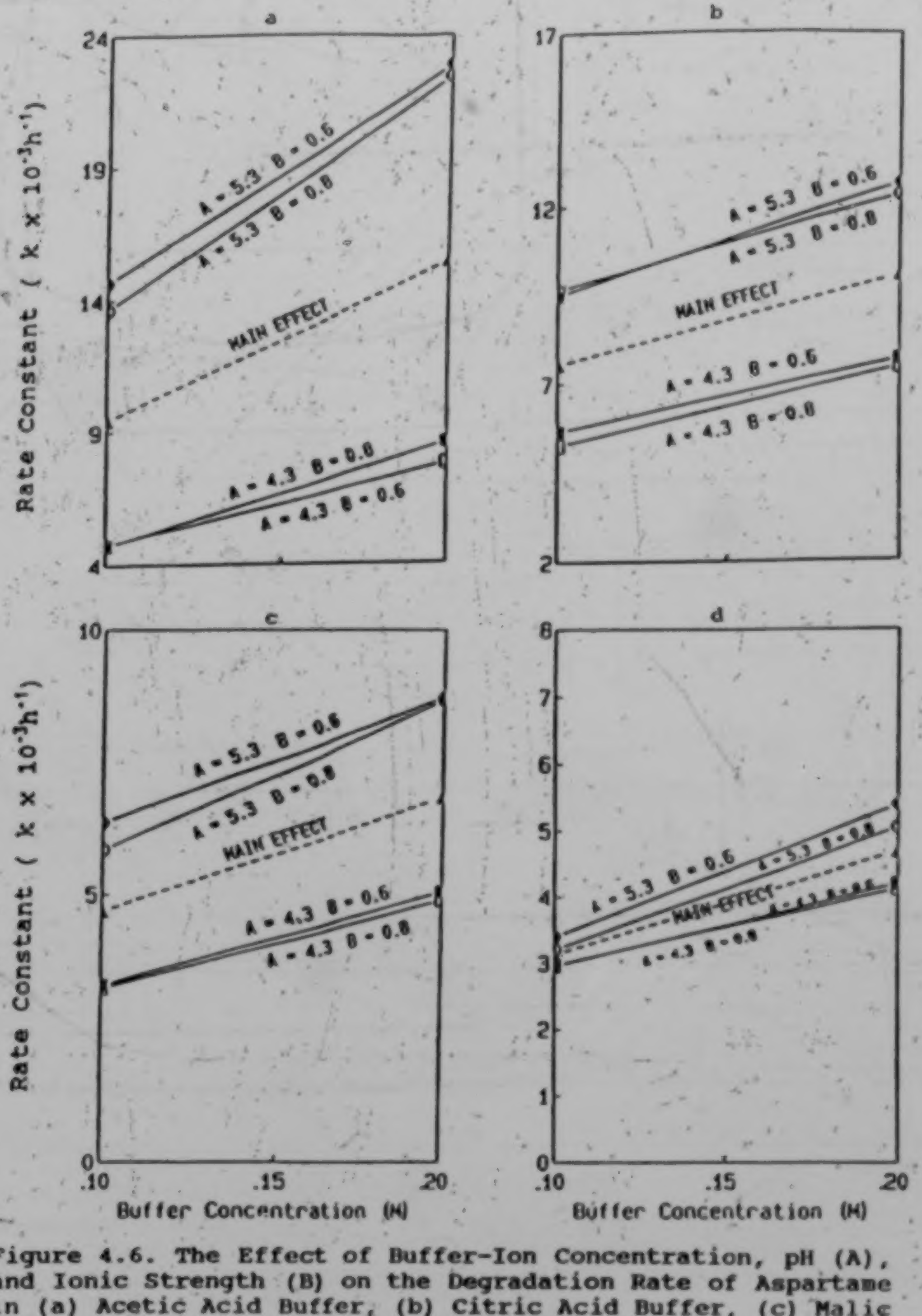


Figure 4.6. The Effect of Buffer-Ion Concentration, pH (A), and Ionic Strength (B) on the Degradation Rate of Aspartame in (a) Acetic Acid Buffer, (b) Citric Acid Buffer, (c) Malic Acid Buffer and, Tartaric Acid Buffer at 60°C.

systems and at the three temperatures studied.

There is a siginificant interaction, ac, between pH and buffer concentration. This interaction can be assessed visually by comparing the slopes of the lines labeled "B = 0.6 C = 0.1" to that of lines "B = 0.6 C = 0.2" as well as comparing the slopes of lines labeled "B = 0.8 C = 0.1" and "B = 0.8 C = 0.2", in Figure 4.4. This same interaction can again be assessed by comparing the slopes of lines labeled "A = 4.3, B = 0.6" to lines labeled "A = 5.3, B = 0.6" as well as comparing the slopes of lines "A = 4.3, B = 0.8" to lines "A = 5.3, B = 0.8 " in Fig. 4.6. The fact that these lines are not parallel means that the effect of pH is dependent on buffer concentration and vice versa.

The effect of temperature can be seen by comparing the rate constants at 60°C and those at 40°C for each buffer system used. When temperature is increased from 40°C to 60°C, the rate constants for all buffers studied increased 5 to 10 fold. The effect of temperature was not tested statistically for significance because temperature was not included, as a factor, in the factorial design used in this study. The temperature dependence of APM degradation, in 0.1 M malic acid buffer at pH 3.0 and ionic strength 0.6, is shown in the form of an Arrhenius plot in Figure 4.7. The natural logarithm of the rate constants of APM degradation was plotted against the reciprocal of the absolute temperatures according to the

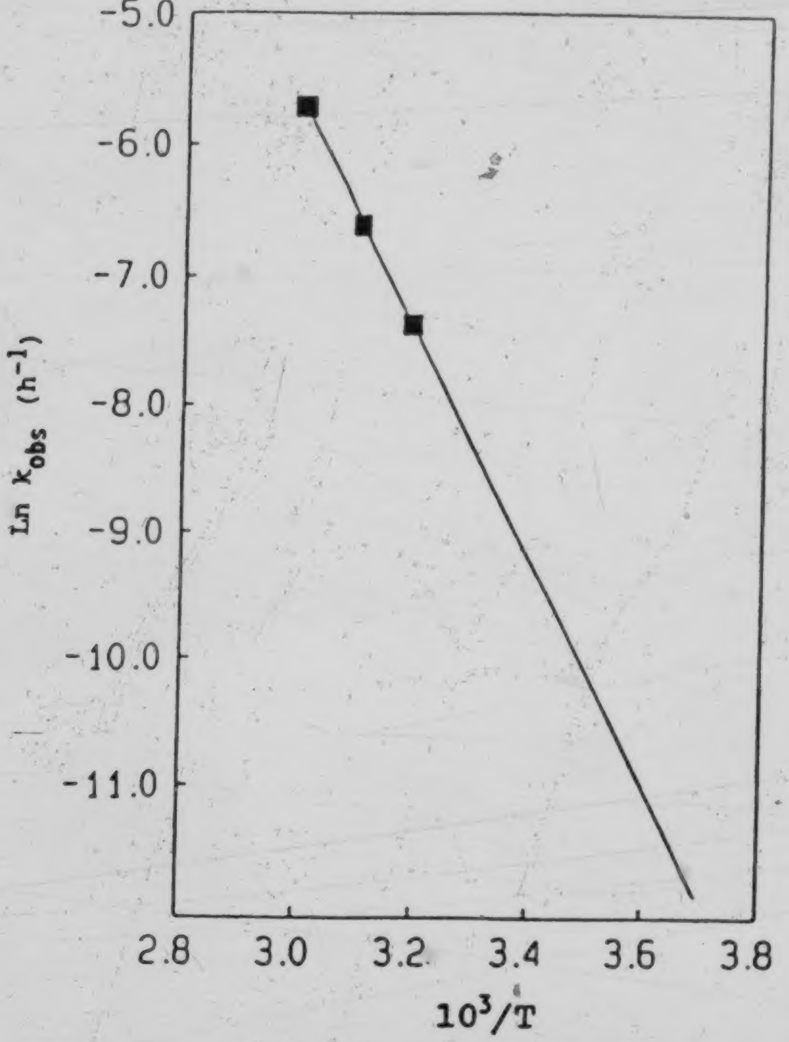


Figure 4.7. Arrhenius Plot of the Degradation of Aspartame in 0.1 M Malic Acid Buffer Solution at pH 3.0.

following form of Arrhenius equation:

$$\ln k = \ln A - E_a/RT \qquad (10)$$

where k is the rate constant of the reaction; A is the frequency factor; E_a is the activation energy of the reaction; R is the gas constant; and T is the absolute temperature. The underlying assumption of Arrhenius equation is that the reaction mechanism does not change as a function of temperature (i.e., E_a is independent of T) (69). From the slope of Fig. 4.7 the activation energy of APM degradation was calculated and a value of 17.864 kcal/mol was obtained. Extrapolation of the graph to 22°C gave a rate constant of APM degradation of 9.91×10^{-5} h^{-1} and a half-life of 291.3 days.

From these experiments one can conclude that increases in pH, buffer concentration, and temperature increased the degradation rate constant of aspartame and that these effects were significant. The interaction of pH and buffer concentration was also significant. Ionic strength did not have a significant effect on the rate constant and it did not interact with pH or buffer concentration. The degradation rate constant of aspartame in the buffer systems used in this study, at all three temperatures, decreased in the following order: acetic acid buffer > citric acid buffer > malic acid buffer > tartaric acid buffer. From this study it can be seen that the aqueous stability of aspartame is enhanced by using minimum buffer concentration, adjusting the pH to 4.3, and using of either malic or tartaric acid buffers.

The use of accelerated stability testing has been used by pharmaceutical scientists in predicting the room temperature stability of drugs. However, there are limitations. Predictions of drug stability room temperature from studies of higher temperature rates of degradation are based on the Arrhenius equation (equation 10). There are a number of situations in which Arrhenius predictions can be erroneous or invalid (133). The Arrhenius equation assumes that the reaction mechanism does not change as a function of temperature. Therefore when different degradation mechanisms predominate at different temperatures, the Arrhenius equation may not be adhered to, and prediction of room temperature stability, becomes marginal at best. At higher temperatures oxygen solubility decreases, consequently the predictability of the room temperature stability of drugs sensitive to oxygen is hindered. For disperse systems, viscosity is decreased as temperature is increased, also in suspensions there is a possibility that the solid phase which exists at room temperature may be different from the solid phase that may exist at an elevated temperature. Alteration of such physical characteristics of the dosage form and drug may result in potentially large errors in predicting room temperature stability. At higher temperatures solvents may evaporate. This produces different solvent compositions at different temperatures which may hinder prediction of room temperature stability (156). Because of the limitations of accelerated stability testing, room temperature stability studies are usually carried out to complement the accelerated stability studies.

4.2 FURTHER ELUCIDATION OF THE DEGRADATION OF ASPARTAME IN AQUEOUS SOLUTION

Figures 4.8, 4.9, and 4.10 illustrate how the degradation of APM is influenced by pH. It can be seen that APM degraded faster at pH 5.3, followed by pH 3.0 and least at pH 4.3. These three figures also show that the major degradation product of APM, in the pH range 3.0 to 5.3, is diketopiperazine. Some of the degradation products such as aspartic acid and methanol were not determined using the HPLC method.

The degradation of aspartylphenylalanine in malic acid buffer solutions is shown in Figures 4.11, 4.12, and 4.13. It can be seen that aspartylphenylalanine degraded least at pH 5.3, Figure 4.11, followed by samples at pH 4.3, the most degradation was observed at pH 3.0, see Figure 4.13. At pH values of 4.3 and 5.3, the amount of diketopiperazine and the amount of phenylalanine formed, from aspartylphenylalanine, was small and almost the same.

This study suggests that the major route of APM degradation, in the pH range examined, is the cyclization

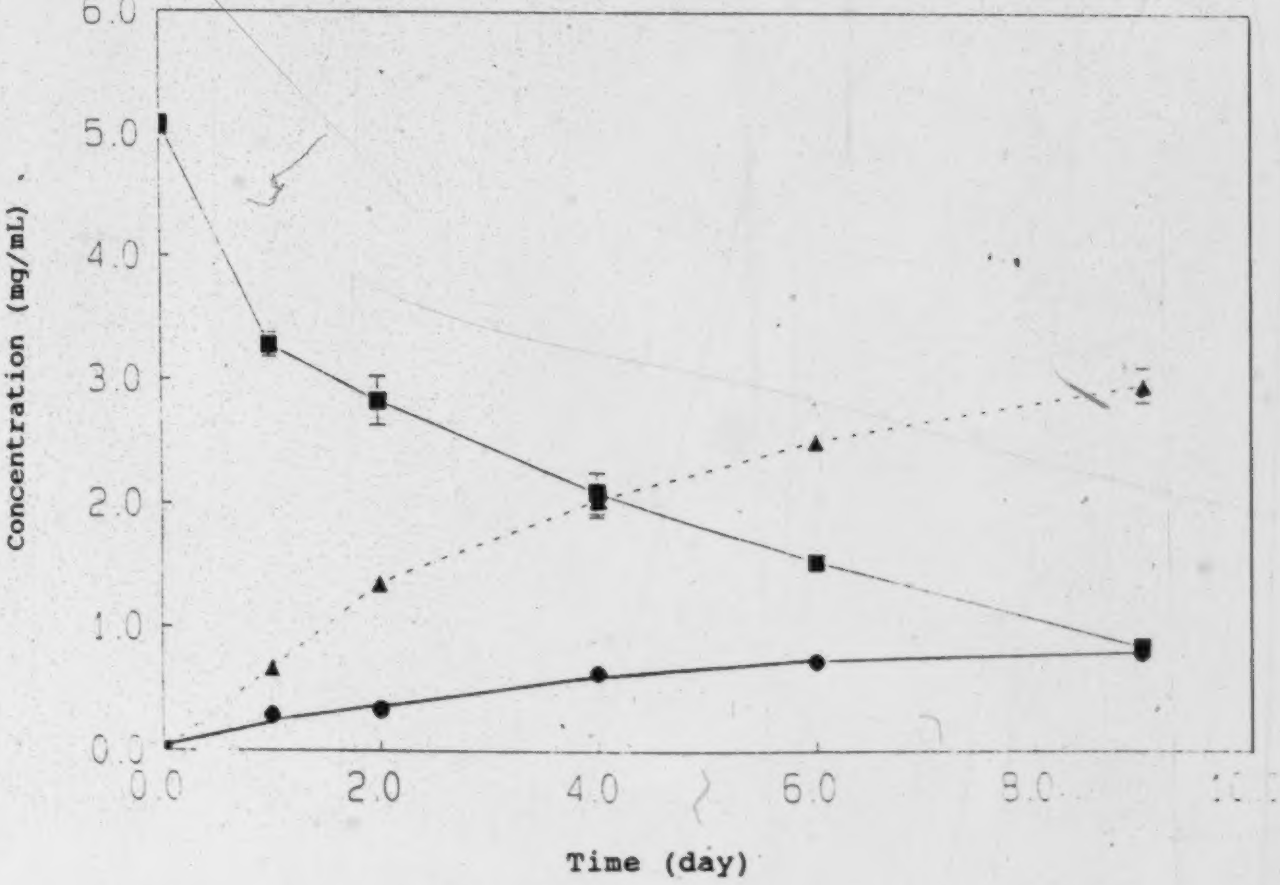


Figure 4.8. Degradation of Aspartame (■) and Formation of Aspartylphenylalanine (●) and Diketopiperazine (▲) in 0.05 M Malic Acid Buffer Solution at pH 5.3 and 60°C.

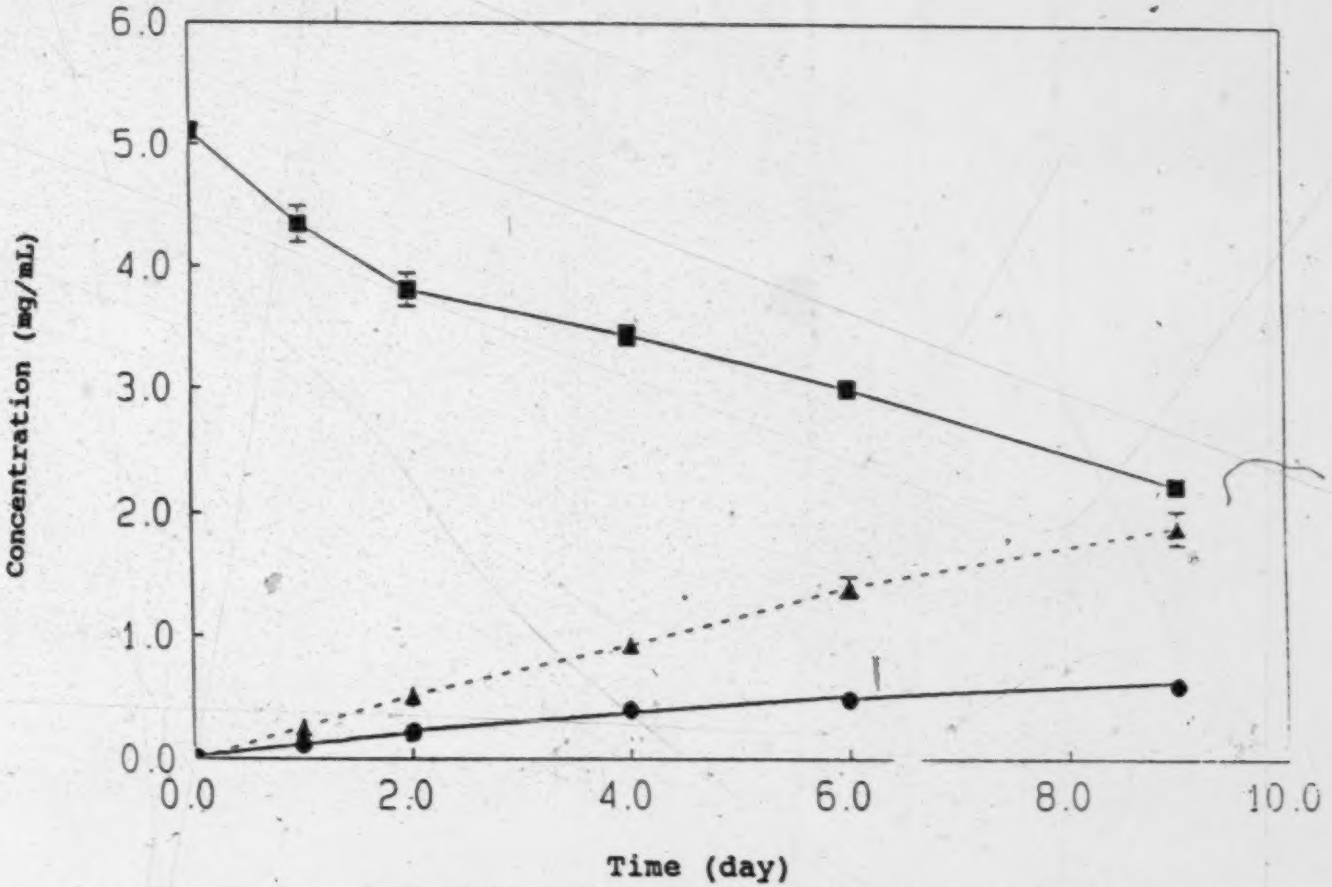


Figure 4.9. Degradation of Aspartame (■) and Formation of Aspartylphenylalanine (●) and Diketopiperazine (▲) in 0.05 M Malic Acid Buffer Solution at pH 4.3 and 60°C.

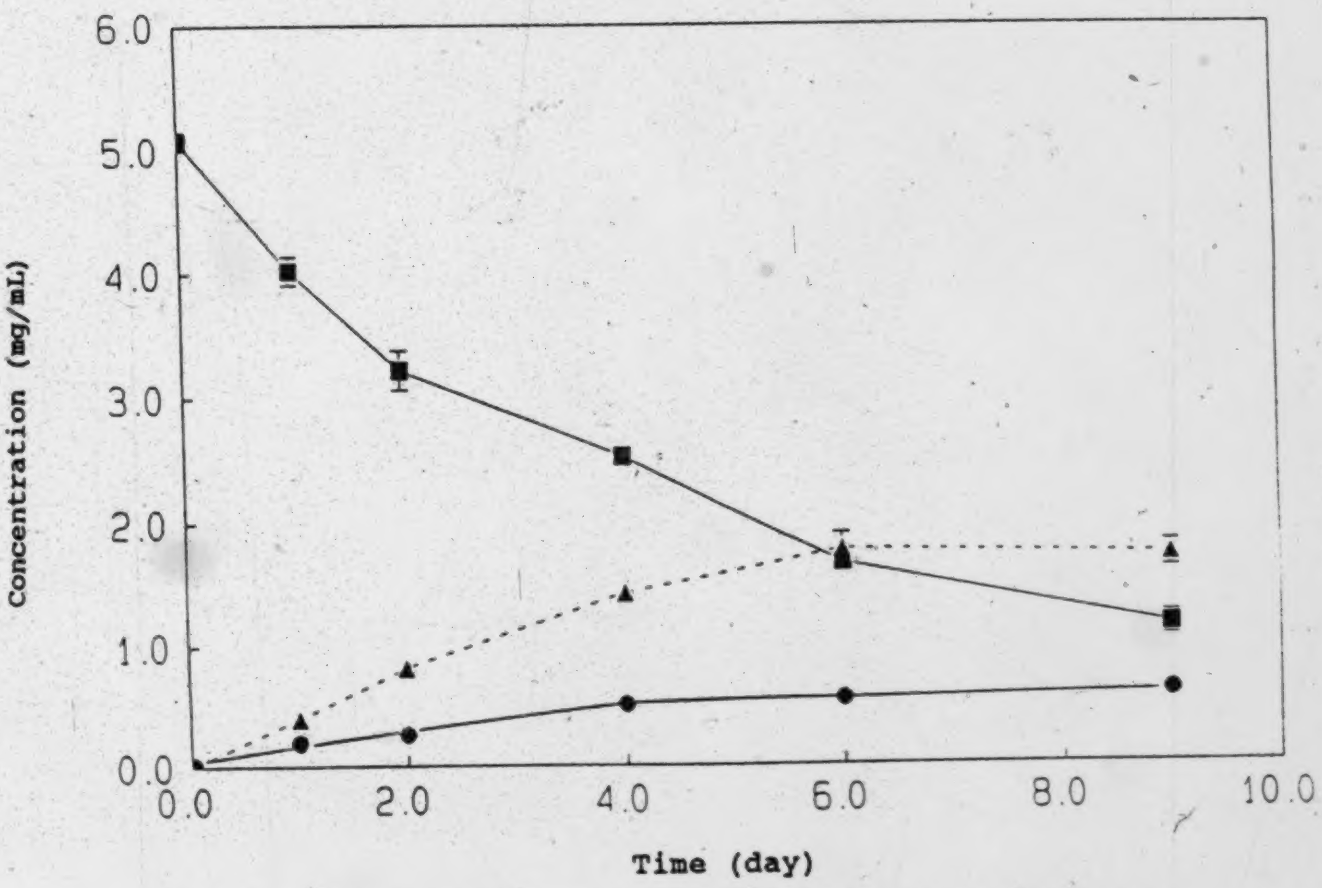


Figure 4.10. Degradation of Aspartame (■) and Formation of Aspartylphenylalanine (●) and Diketopiperazine (▲) in 0.05 M Malic Acid Buffer Solution at pH 3.0 and 60°C.

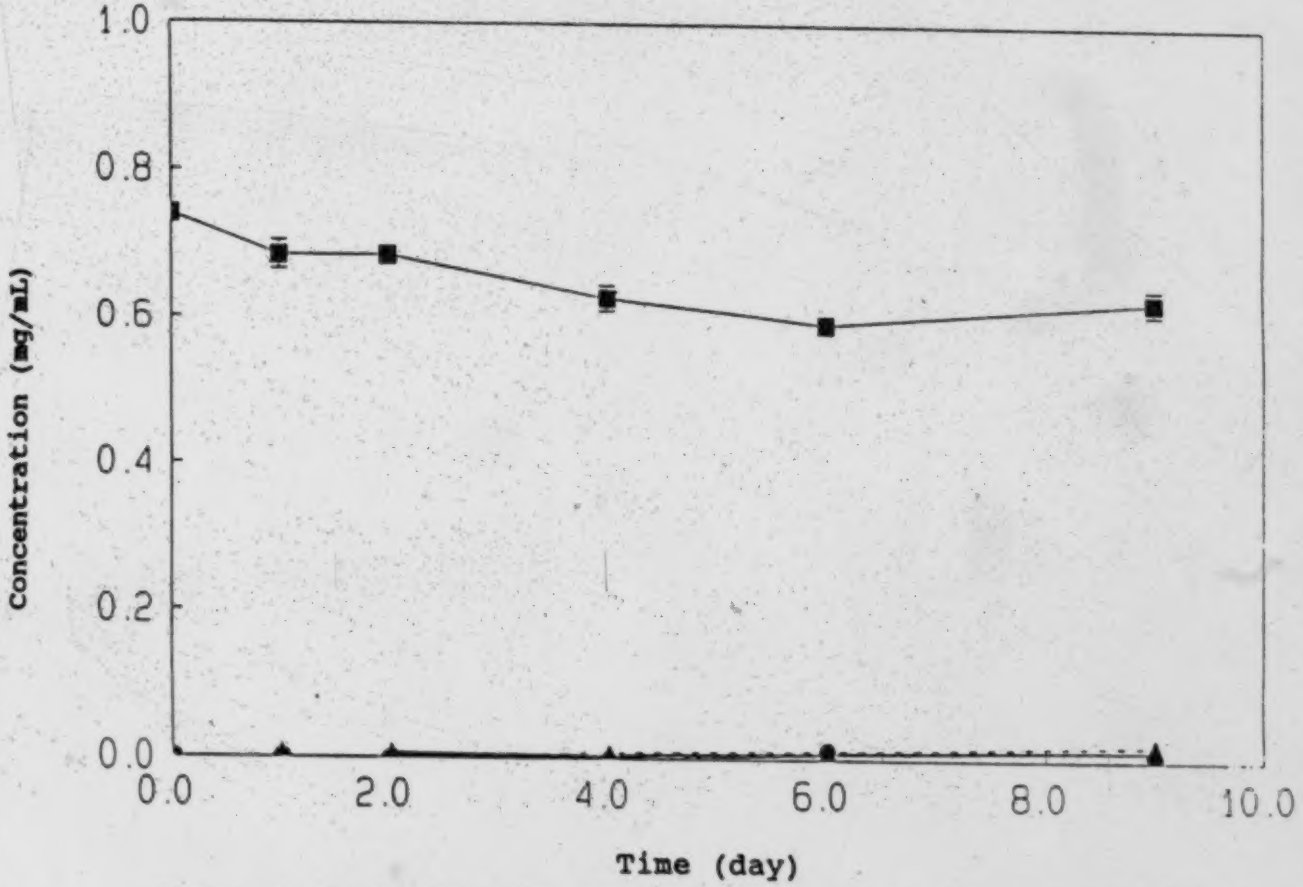


Figure 4.11. Degradation of Aspartylphenylalanine (■) and Formation of Diketopiperazine (▲) and Phenylalanine (●) in 0.05 M Malic Acid Buffer Solution at pH 5.3 and 60°C.

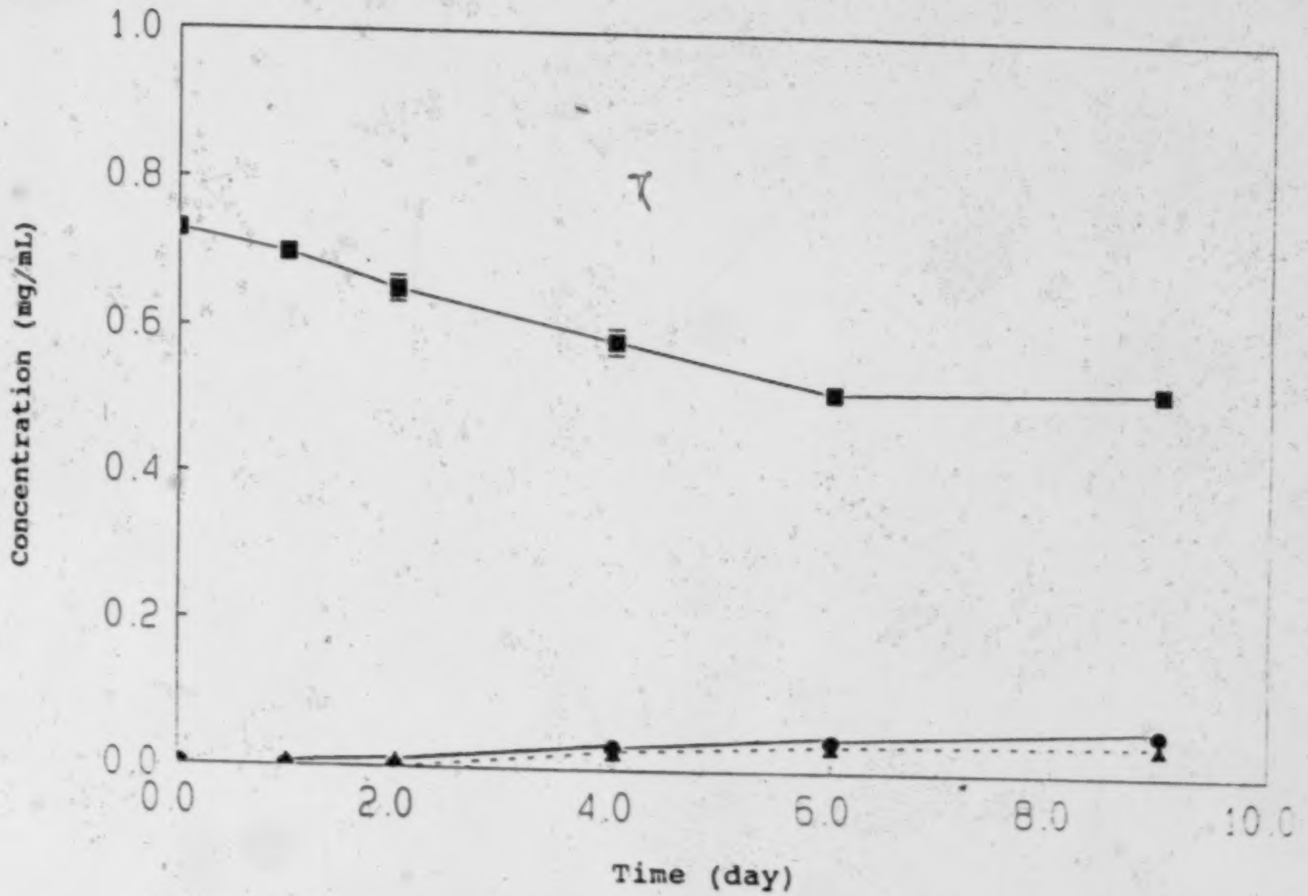


Figure 4.12. Degradation of Aspartylphenylalanine (■) and Formation of Diketopiperazine (▲) and Phenylalanine (●) in 0.05 M Malic Acid Buffer Solution at pH 4.3 and 60°C.

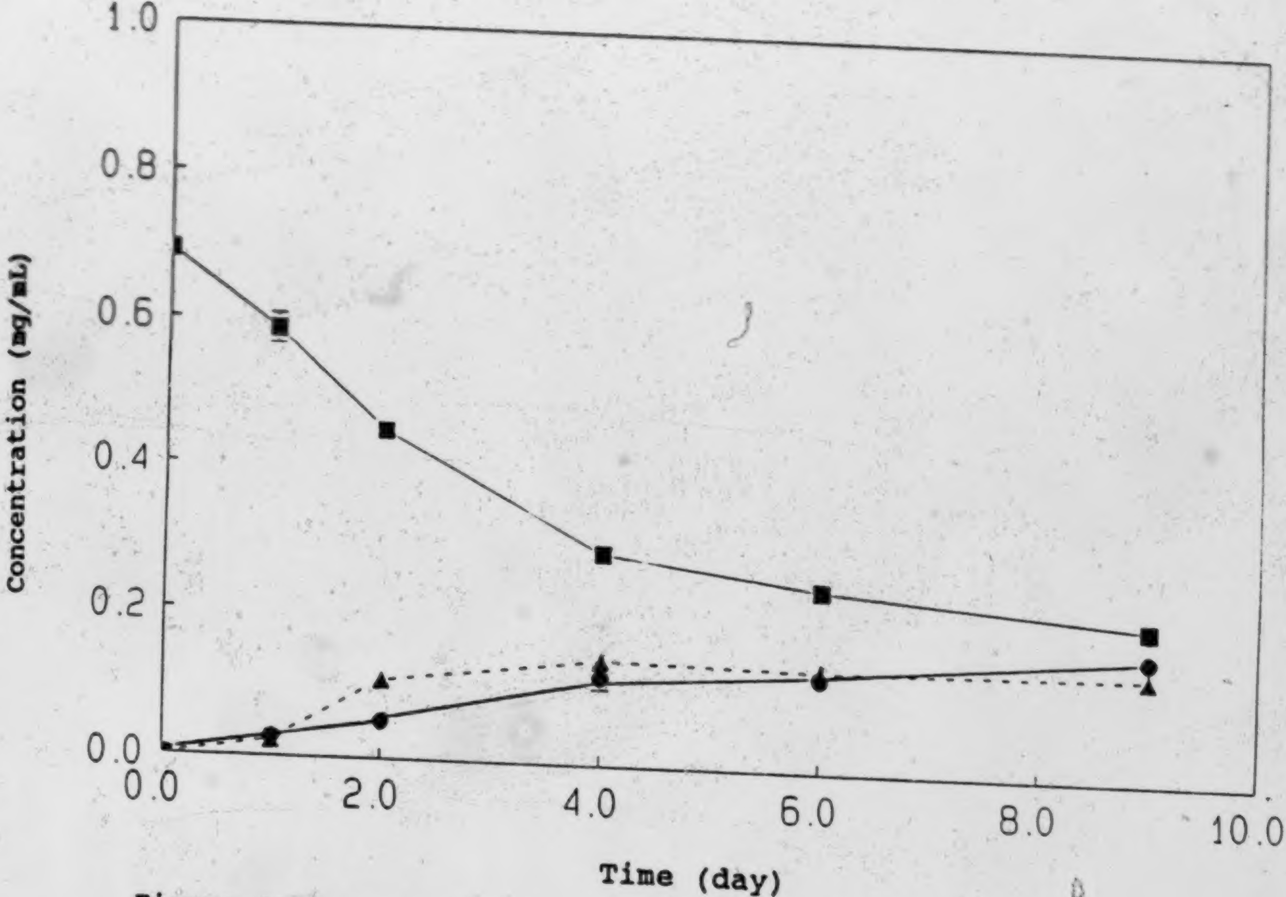


Figure 4.13. Degradation of Aspartylphenylalanine (■) and Formation of Diketopiperazine (▲) and Phenylalanine (●) in 0.05 M Malic Acid Buffer Solution at pH 3.0 and 60°C.

reaction to DKP. This is indicated by the fact that DKP is the major degradation product of APM at all the pH levels used in this study. Also this suggestion is supported by the observation that there is very little formation of DKP from aspartylphenylalanine.

4.2.1 Specific and General Acid-Base Catalysis

The degradation of APM in buffered aqueous solution is subject to specific and general acid-base catalysis. Specific acid catalysis is catalysis by the solvated protons, that is, by the hydronium ion in aqueous solution. Specific base catalysis is catalysis by the hydroxide ions in aqueous solution. General acid catalysis is catalysis by a proton acid other than the hydronium ion. A general acid catalyst is hence a Bronsted acid. General base catalysis is catalysis by a Bronsted base, other than by hydroxide ion acting as a proton acceptor, that is, by sharing an electron pair with a proton (134).

From the classical theory of catalysis by hydrogen and hydroxide ions, at zero buffer-ion concentration, the most general expression for the observed rate constant, k_{obs}, is (135,136):

$$k_{obs} = k_o + k_H + [H^+] + k_{OH}[OH^-] \qquad (11)$$

where k_o is the rate constant of "spontaneous" reaction, k_H is the hydrogen ion catalytic rate constant and k_{OH} is the hydroxide ion catalytic rate constant. The dissociation of water is given by:

$$K_w = [H^+][OH^-] \tag{12}$$

When the ion product of water, equation 12, is substituted into equation 11, the following two equations result (135,136):

$$k_{obs} = k_o + k_H[H^+] + \frac{k_{OH}\ Kw}{[H^+]} \tag{13}$$

$$k_{obs} = k_o + \frac{k_H\ K_w}{[OH^-]} + k_{OH}[OH^-] \tag{14}$$

It can be seen from equations 13 and 14 that there are two ranges where the observed rate constant is a linear function of $[H^+]$ and $[OH^-]$. In acid solution, where the hydrogen-ion concentration is very high, the third term in equation 13 is negligible, the observed rate constant is given by:

$$k_{obs} = k_o + k_H[H^+] \tag{15}$$

Taking the logarithms of equation 14, we get (137):

$$\log k_{obs} = \log k_o + \log k_H + \log[H^+] \tag{16}$$

or equivalently,

$$\log k_{obs} = \log k_o + \log k_H - (-\log [H^+]) \qquad (17)$$

which can be written as

$$\log k_{obs} = \log k_o + \log k_H - pH \qquad (18)$$

Ploting log k_{obs} versus pH of the solution gives a line of slope equal to -1. Also in alkaline solution when the hydroxide-ion concentration is very high, the second term in equation 14 is negligible and the observed rate constant is given by

$$k_{obs} = k_o + k_{OH}[OH^-] \qquad (19)$$

Substituting equation 12 in equation 19, we get

$$k_{obs} = k_o + \frac{k_{OH} K_w}{[H^+]} \qquad (20)$$

Taking the logarithms of equation 20, we get:

$$\log k_{obs} = \log k_o + \log k_{OH}K_w - \log [H^+] \qquad (21)$$

Equation 21 can be written as:

$$\log k_{obs} = \log k_o + \log k_{OH}K_w + pH \qquad (22)$$

In this case, a plot of log k_{obs} against pH should be linear with a slope equal to +1 (137).

Between these two extreme regions the observed rate constant passes through a minimun at a hydrogen-ion concentration given by (135,136):

$$[H^+] = \left(\frac{k_{OH}K_w}{[H^+]}\right)^{1/2} \qquad (23)$$

If $k_{OH} = k_H$, the minimum observed rate constant will be at the neutral point, pH 7. The other two possibilities are $k_{OH} > k_H$ and $k_{OH} < k_H$, which correspond to a minimum observed rate constant on the acid and on the alkaline side of the neutral point, respectively. The observed rate constant at minimum point, k_{min}, is given by (135,136):

$$k_{min} = k_o + 2(K_w k_H k_{OH})^{1/2} \qquad (24)$$

When the "spontaneous" rate constant is large, that is, $k_o > (K_w k_H k_{OH})^{1/2}$, the pH-rate profile will have a horizontal portion in the region of minimum velocity. The rate of the reaction in this horizontal portion of the pH-rate profile is pH independent. If the "spontaneous" rate constant is small, that is, $k_o < (K_w k_H k_{OH})^{1/2}$, the slope of the pH-rate profile in the region of minimum velocity changes. The horizontal portion disappears and is replaced by a fairly sharp minimum. Because the second term in equation 24 is not negligible, the "spontaneous" reaction rate constant, k_o, cannot be directly observed (135,136).

4.2.2 Catalytic Effect of Buffers

In addition to the specific acid and specific base catalysis described in the preceding section, APM, in aqueous solutions of the four buffer systems used in this study, is subject to general acid and general base catalysis. Statistical analysis of data in Tables 4.2 to 4.4 and in Tables A.2 to A.13 indicate that the observed rate constant increased significantly when the total concentration of buffer-ions was increased from 0.1 M to 0.2 M. The observed rate constant in citric acid buffer, for example, was actually a summation of several catalytic rate constants catalyzed by the buffer species plus the rate constant at zero buffer-ion concentration. The observed rate constant may be expressed by the following equation (138,139):

$$k_{obs} = k_o + k_H[H^+] + k_1[H_3A] + k_2[H_2A^-] + k_3[HA^{2-}] + k_4[A^{3-}] + k_{OH}[OH^-] \qquad (25)$$

where $k_o + k_H + [H^+] + k_{OH}[OH^-]$ is the expression of the rate constant at zero buffer-ion concentration; other rate constants are catalytic rate constants imposed by the citrate buffer species ; $[H_3A]$ is the concentration of the undissociated citric acid; $[H_2A^-]$ is the concentration of the dihydrogen citrate ion; $[HA^{2-}]$ is the concentration of the monohydrogen citrate ion; and $[A^{3-}]$ is the concentration of the citrate ion. The total citrate buffer-ion concentration, C_A, is given by:

$$C_A = [H_3A] + [H_2A^-] + [HA^{2-}] + [A^{3-}] \qquad (26)$$

This is from the dissociation constants

$$K_1 = \frac{[H^+][H_2A^-]}{[H_3A]} \qquad (27)$$

$$K_2 = \frac{[H^+][HA^{2-}]}{[H_2A^-]} \qquad (28)$$

$$K_3 = \frac{[H^+][A^{3-}]}{[HA^{2-}]} \qquad (29)$$

From equation 25 and 26 the following overall rate expression, k_{obs}, was obtained (139):

$$k_{obs} = k_o + k_H[H^+] + k_{OH}[OH^-] + C_A\left(\frac{k_1[H^+]^3 + k_2[H^+]^2K_1 + k_3[H^+]K_1K_2 + k_4K_1K_2K_3}{[H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3}\right) \tag{30}$$

Based on the pK_a values; $pK_1 = 3.11$, $pK_2 = 4.75$, and $pK_3 = 6.42$ (139) and the pH, the relative amounts of each of the citrate buffer species were calculated.

At pH 4.3 there are about 4% undissociated citric acid molecules, 64% dihydrogen citrate ions and 31% monohydrogen citrate ions. At this pH, equation 26, which is any expression of the total citrate buffer-ion concentration may be simplified to;

$$C_A = [H_3A] + [H_2A^-] + [HA^{2-}] \tag{31}$$

Also the observed rate constant can be simplified to;

$$k_{obs} = k_o + k_H[H^+] + k_{OH}[OH^-] + C_A\left(\frac{k_1[H^+]^2 + k_2[H^+]K_1 + k_3K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2}\right) \tag{32}$$

A similar analysis can be done at pH 5.3. There are 16% dihydrogen citrate ions, 78% Monohydrogen citrate ions and 6% citrate ions. Accordingly, the total citrate buffer-ion concentration and the overall observed rate constant may also be simplified to:

$$C_A = [H_2A^-] + [HA^{2-}] + [A^{3-}] \tag{33}$$

$$k_{obs} = k_o + k_H + [H^+] + k_{OH}[OH^-] + C_A \left(\frac{k_2[H^+]^2 + k_3[H^+]K_2 + k_4K_2K_3}{[H^+]^2 + [H^+]K_2 + K_2K_3} \right) \tag{34}$$

This analysis was done for each of the four buffer systems used. In acetic acid buffer solution, at pH 4.3, there was about 70% undissociated acetic acid molecules, and 30% acetate ions. At pH 5.3, 20% of acetic acid molecules were undissociated and 80% of the acetic acid molecules were dissociated. For malic acid buffer, at pH 4.3, there were 10% undissociated malic acid molecules, 77% monohydrogen malate ions and 13% malate ions. At pH 5.3, there were 36% monohydrogen malate ions and 64% malate ions. For tartaric acid buffer at pH 4.3, there were 2% undissociated tartaric acid molecules, 46% monohydrogen tartarate ions and 52% tartarate ions. At pH 5.3 there were about 8% monohydrogen tartarate ions and 92% tartarate ions.

The diagrams in Fig. 4.6 are plots of the observed rate constants against total buffer concentration of the simplified rate expressions like those given in equations 32 and 34. The ionic strength was kept constant at 0.6 or 0.8. The slopes of these linear plots are given by expressions similar to following:

$$\frac{k_2[H^+]^2 + k_3[H^+]K_2 + k_3K_2K_3}{[H^+]^2 + [H^+]K_2 + K_2K_3}$$

in equation 34 and the intercept is equal to

$$k_o + k_H[H^+] + k_{OH}[OH^-].$$

Tables 4.2 to 4.4 and Tables A.2 to A.13 indicate that the effect of pH and buffer-ion concentration significantly increased the rate constant of APM degradation. The effect of pH on the observed rate constant is actually the effect contributed by the following rate constants, $k_o + k_H[H^+] + k_{OH}[OH^-]$. This is the observed rate constant when the buffer-ion concentration is zero. The effect of buffer-ion concentration on the observed rate constant is the effect contributed by an expression similar to the following;

$$C_A \frac{k_2[H^+]^2 + k_3[H^+]K_2 + k_4K_2K_3}{[H^+]^2 + [H^+]K_2 + K_2K_3}$$

in equation 34. Tables 4.2 to 4.4 and Tables A.2 to A.13 indicate that the magnitude of the calculated effect of pH, on the observed rate constant, for acetic acid buffer, citric acid buffer, and malic acid buffer is larger than the calculated effect of the buffer-ion concentration. But for tartaric acid buffer, it can be seen that the magnitude of the calculated effect of the buffer-ion concentration is larger than the calculated effect of pH on the observed rate constant. This supports the contention that the acetic acid buffer, citric acid buffer, and malic acid buffer systems showed specific and general acid-base catalysis whereas tartaric acid buffer showed only general acid-base catalysis.

4.2.3 The Salt Effect

There are two salt effects on the rate of chemical reaction in aqueous solution (140). The primary salt effect is caused by change in the activities of the ions in solution brought about by the addition of neutral salts like sodium chloride. The primary salt effect is evaluated by changing the ionic strength of the solution, holding other factors such as pH, solute concentration, and others constant, and determining if the rate constant changes. In most pharmaceutical liquid dosage forms, the ionic strength is usually relatively high because of the buffers, preservatives, and chelating agents which might be required components of the formula. Lewis introduced the concept of ionic strength in order to relate interionic attraction to activity (141). The activity of an ion in solution of strong electrolyte is generally less than the actual or stoichiometric concentration of the solute. This is because some of the ions are effectively "taken out of play" by the electrostatic forces of interaction (141). Ionic strength, I, can be defined as (141):

$$I = \tfrac{1}{2}\, \Sigma c_i z_i 2 \qquad (35)$$

where c_i is the concentration in moles/liter of any ions in solution and z_i is the ion's valence. By varying the ionic strength, the rate constant of the reacting species will either increase, remain constant or decrease. The relationship of ionic strength to the rate constant of a reaction is described by the Bronsted-Bjerrum equation (142,143):

$$\log k_{obs} = \log k_o + 2Qz_A z_B (I)^{\frac{1}{2}} \qquad (36)$$

where k_{obs} is the observed rate constant at ionic strength, I, 2Q is a constant equal to 1.02 at 25°C, and z_A, z_B are charges of the reacting species. The relatioship expressed in equation 25, between rate constant and ionic strength is usually obeyed in ionic strength ranges up to 0.01 (146) Carstensen proposed a modification of the Bronsted-Bjerrum equation based on the modified Debye-Huckel equation 36 (144):

$$\log k_{obs} = \log k_o + 2Qz_A z_B ((I)^{\frac{1}{2}}/1+(I)^{\frac{1}{2}}) \qquad (37)$$

This equation has been used for solutions at high ionic strength. The charges z_A and z_B determine whether the rate constant will increase, remain constant or decrease with changes in the ionic strength. If z_A and z_B are like-sign ions then the rate constant will increase with an increase in ionic strength. If z_A and z_B are different-sign ions, then an increase in ionic strength will result in a decrease in the rate constant of the reaction. Lastly, if the charge on either z_A or z_B is zero, then the rate constant will not be affected by changes in ionic strength. The results of APM degradation, in buffered aqueous solutions, indicated that ionic strength did not significantly affect the rate constant. The primary salt effect on this reaction was small which could be due to the fact that the range of ionic strength studied was small. The results suggest that as the ionic strength was increased the rate constant of APM degradation decreased but the observed. The decrease in the rate constant was not statistically significant.

The secondary salt effect on the rate of a chemical reaction is brought about by displacement of the equilibrium constant caused by the addition of neutral salts (140). Thus for example considering the equilibrium shown in equation (38), which is catalyzed by hydrogen ions;

$$[A] \rightleftharpoons [B] + [H^+] \qquad (38).$$

If $[H^+]$ is equal to [B], that is a solution containing a weak acid alone, the change in dissociation constant will affect both equally. If [A] is equal to [B], and large compared to $[H^+]$, that is, a buffer solution, then only the hydrogen-ion concentration will be appreciably affected, and for a small change, the effect of the salt concentration will be twice as great as in the previous case. Finally, if [A] is small compared to both [B] and $[H^+]$, that is a solution of a strong acid, $[H^+]$ will be almost unaffected by changes in ionic strength and the secondary salt effect will be zero (140). The secondary salt effect was taken care of, in these studies, by using the following general equation for the calculation of pH (144):

$$pH = pK_{an}+\log([salt]/[acid])-Q([(2n-1)(I)^{\frac{1}{2}}]/[1+(I)^{\frac{1}{2}}]) \tag{39}$$

where K_a is the acid dissociation constant, n is the stage of ionization of the polybasic acid and the other symbols are the same as defined before.

4.2.4 Effect of Temperature on Buffer pH

The change of hydrogen ion activity, a_H, of a buffer solution composed of a weak acid and its salt, with temperature is given by the following equation (145):

$$dpa_H/dT = -d\log K_a/dT - (2z + 1)(d\log y_{Ac-})/dT \qquad (40)$$

For a buffer solution consisting of a weak base and its salt, the hydrogen ion activity is given by (145):

$$dpa_H/dT = -d\log K_w/dT - d\log K_b/dT - (2z+1)(d\log y_{BH+})/dT \qquad (41)$$

where d is partial derivative, y_{Ac-} and y_{BH+} are activities of the salt for the weak acid and the weak base respectively. Bates has shown that the fraction dlog y_{Ac-}/dT usually is very small and has stated that the plots of -log K_a as a function of temperature are similar to the plots of -log a_H versus temperature. These plots are roughly parabolic in form, and for many weak electrolytes the pk_a has a minimum value in the temperature range of 0°C to 60°C. Hence dlog K/dT may be either positive or negative at 25°C. In general the characteristic minimum is displaced towards higher temperatures as the acid strength decreases, so that pK_a increases with temperature for stronger acids and higher temperatures and decreases with temperature for weaker acids and lower temperatures, see Table 4.5 (145). For weak bases, pK_b usually decreases with rising temperatures. The pH of some buffers increases with temperature for example acetic acid, whereas the pH of others, such as boric acid buffer, decreases with temperature. The temperature coefficients of

Table 4.5

Changes in pK_a as Temperature Increases

Acid	pK_a at Temperatures	
	25°C	60°C[a]
Acetic acid	4.78	4.58
Citric acid	3.12 4.76 6.39	2.72 4.30 5.47
Boric acid	9.24	8.75

[a] Data from Per Finholt et al. (138).

acid buffers are relatively small. The pH of most basic buffers have been found to change markedly with temperature owing to K_w, which appears in the equation for the basic buffers and which changes significantly with temperature.

In this study the pH of the buffered aqueous solutions of APM was only determined at room temperature. The error in the results, introduced by this procedure is probably small, because the buffers used were all acid buffers whose pHs are not markedly changed by changes in temperature.

4.3 STABILITY OF ASPARTAME IN MIXED SOLVENT SYSTEMS.

Figures 4.14 to 4.16 show that plots of natural logarithm of concentration of aspartame remaining in the samples versus time. They are linear and have negative slopes which indicates that the decomposition of aspartame in alcohol-water solvent systems, as in buffered aqueous solutions, followed pseudo first-order kinetics. The rate constants presented in Table 4.6 are the mean and standard deviation of the rate constants determined for four samples. Figures 4.17 to 4.19 show that the relationship between natural logarithm of the rate constants of the degradation of aspartame and the reciprocal of the dielectric constant of the media are linear

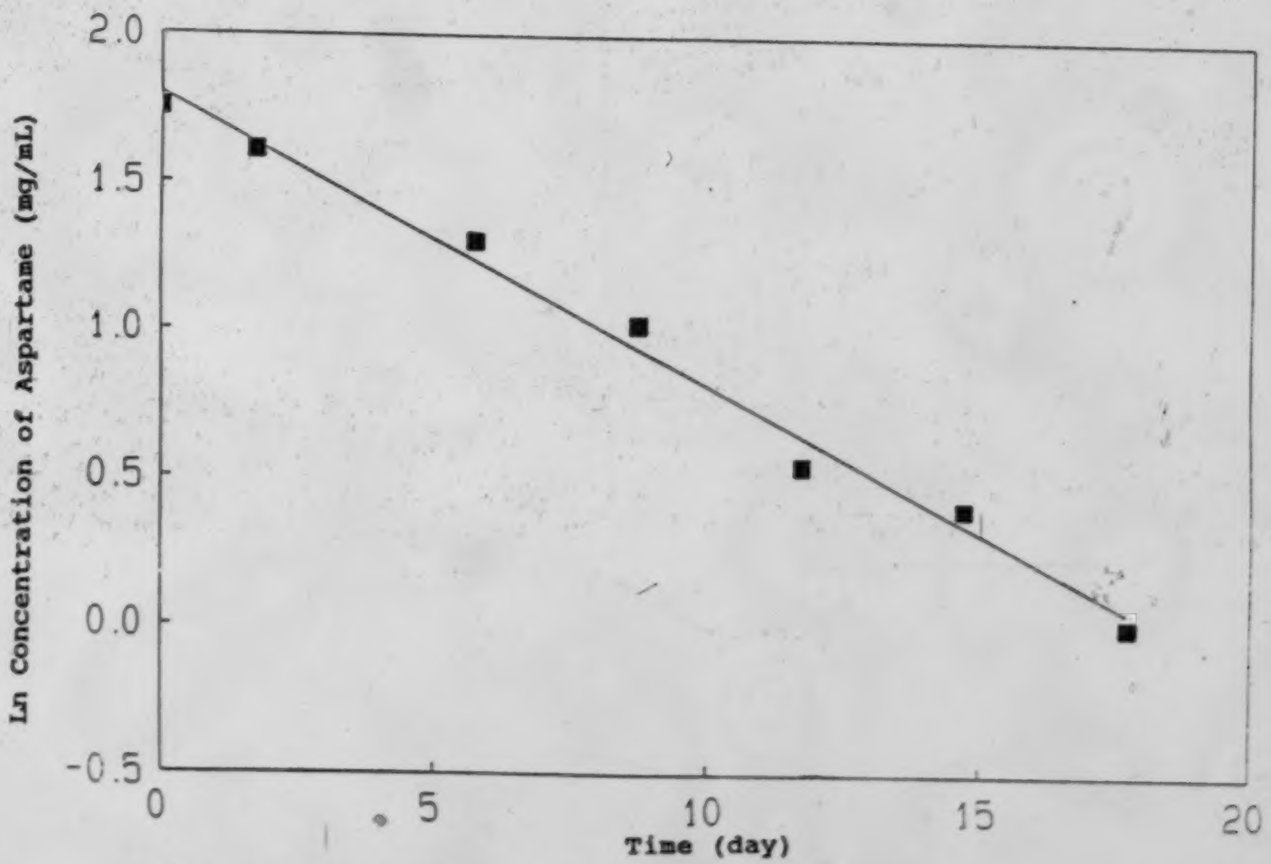


Figure 4.14. Pseudo First Order Degradation of Aspartame in 55.2% Ethanol-Water Mixture at Apparent pH 4.5 and 50°C.

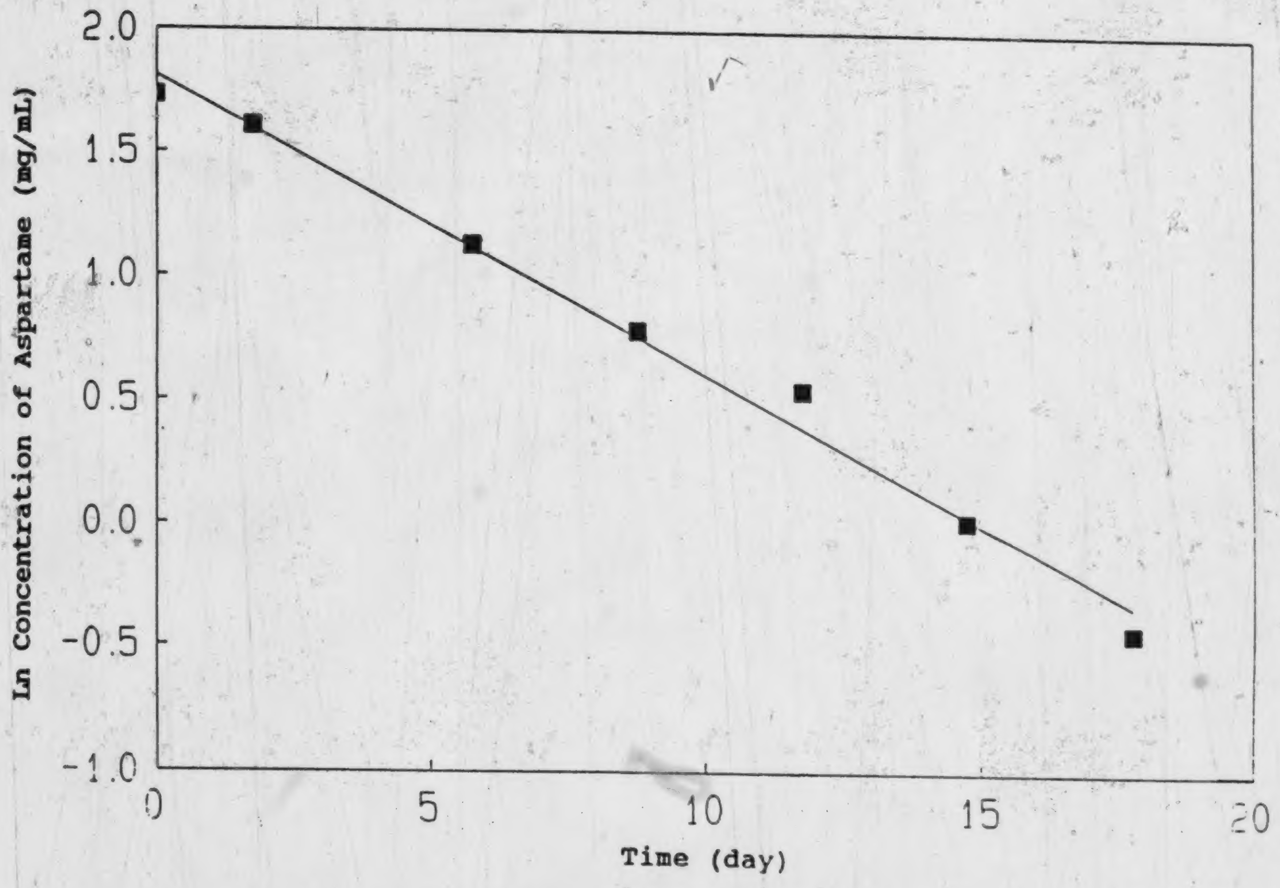


Figure 4.15. Pseudo First Order Degradation of Aspartame in 63.8% Methanol-Water Mixture at Apparent pH 4.5 and 50°C.

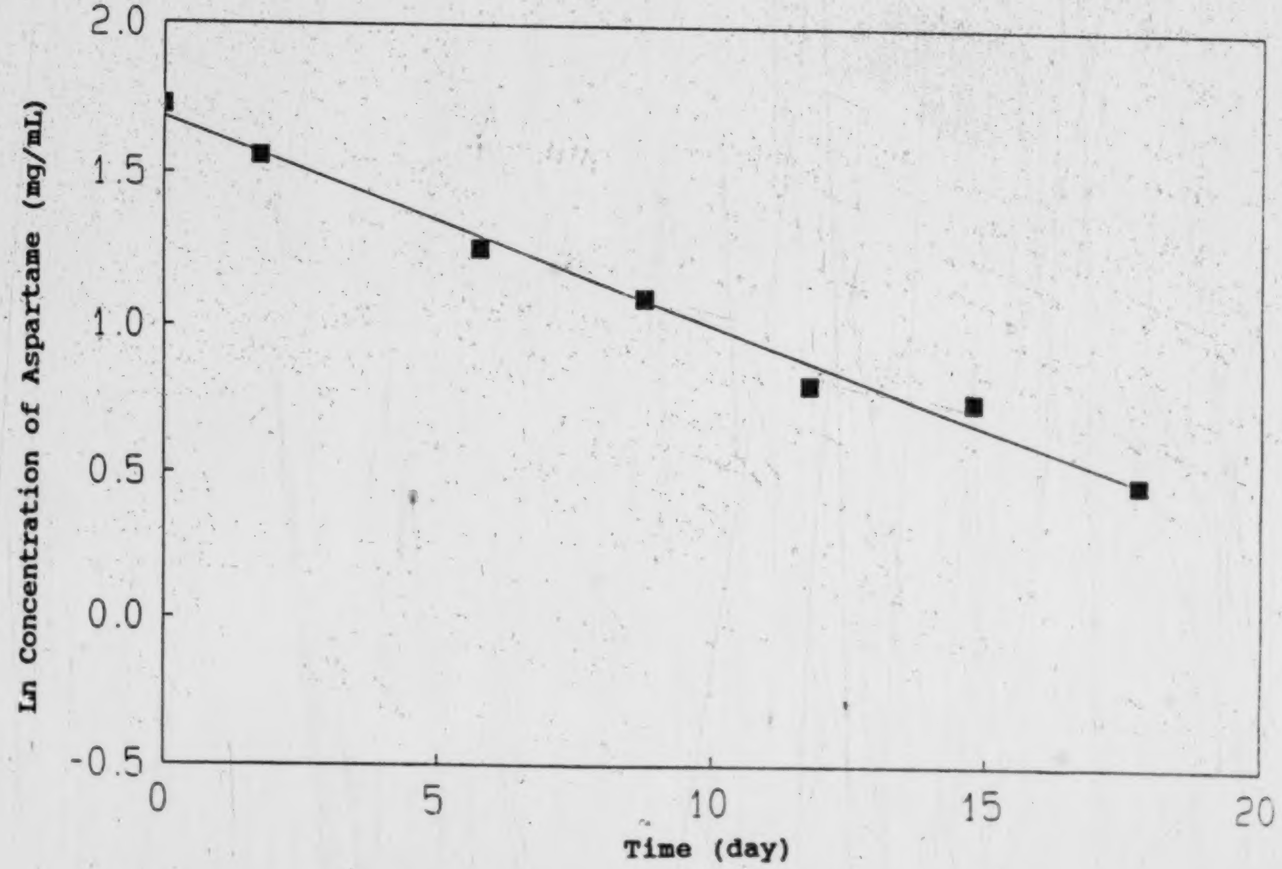


Figure 4.16. Pseudo First Order Degradation of Aspartame in 78.5% Glycerol-Water Mixture at Apparent pH 4.5 and 50°C.

Table 4.6

Stability of Aspartame in Solvent Systems with Different Dielectric Constants

Temperature (°C)	Dielectric Constant	Mean Rate Constant ($k \times 10^{-3} hr^{-1}$) in		
		Water-EtOH	Water-MeOH	Water-Glycerol
60	65	4.36 (.57)[a]	4.10 (.33)	4.81 (.67)
	55	5.55 (.39)	5.93 (.08)	5.74 (.47)
	45	8.63 (.51)	8.84 (.81)	7.96 (.71)
50	65	1.66 (.15)	1.73 (.17)	1.67 (.16)
	55	2.40 (.52)	2.52 (.45)	2.23 (.33)
	45	3.85 (.35)	4.45 (1.12)	2.89 (.71)
40	65	0.82 (.06)	0.82 (.08)	0.74 (.20)
	55	1.25 (.12)	1.34 (.39)	1.00 (.25)
	45	2.17 (.04)	2.08 (.37)	1.41 (.01)

[a] Standard deviation, n = 4.

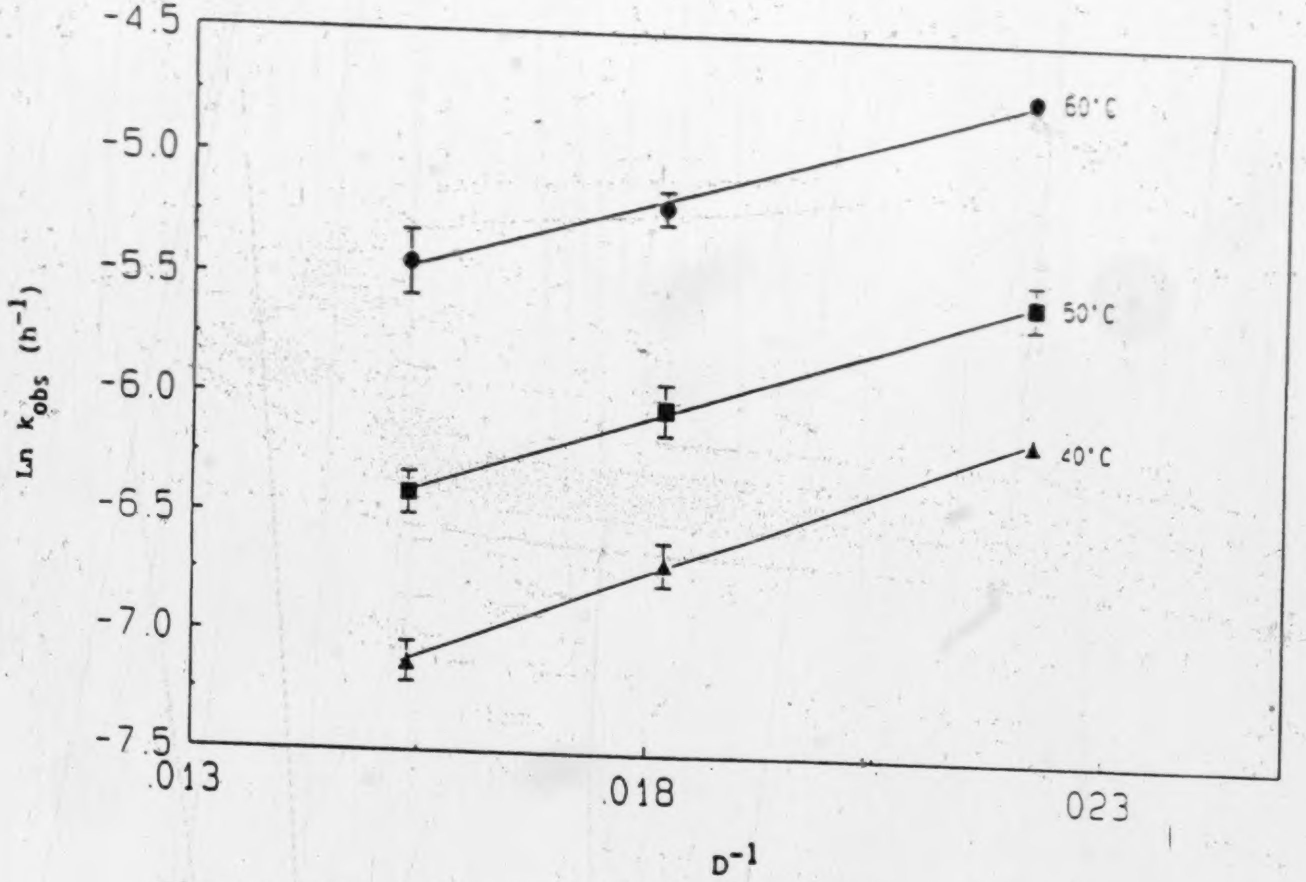


Figure 4.17. Plot of the Observed Rate Constant of Aspartame Degradation Versus the Reciprocal of Dielectric Constant of the Ethanol-Water Solvent Mixture. The Dielectric Constant Values of the Solvent Mixtures were 45, 55, and 65.

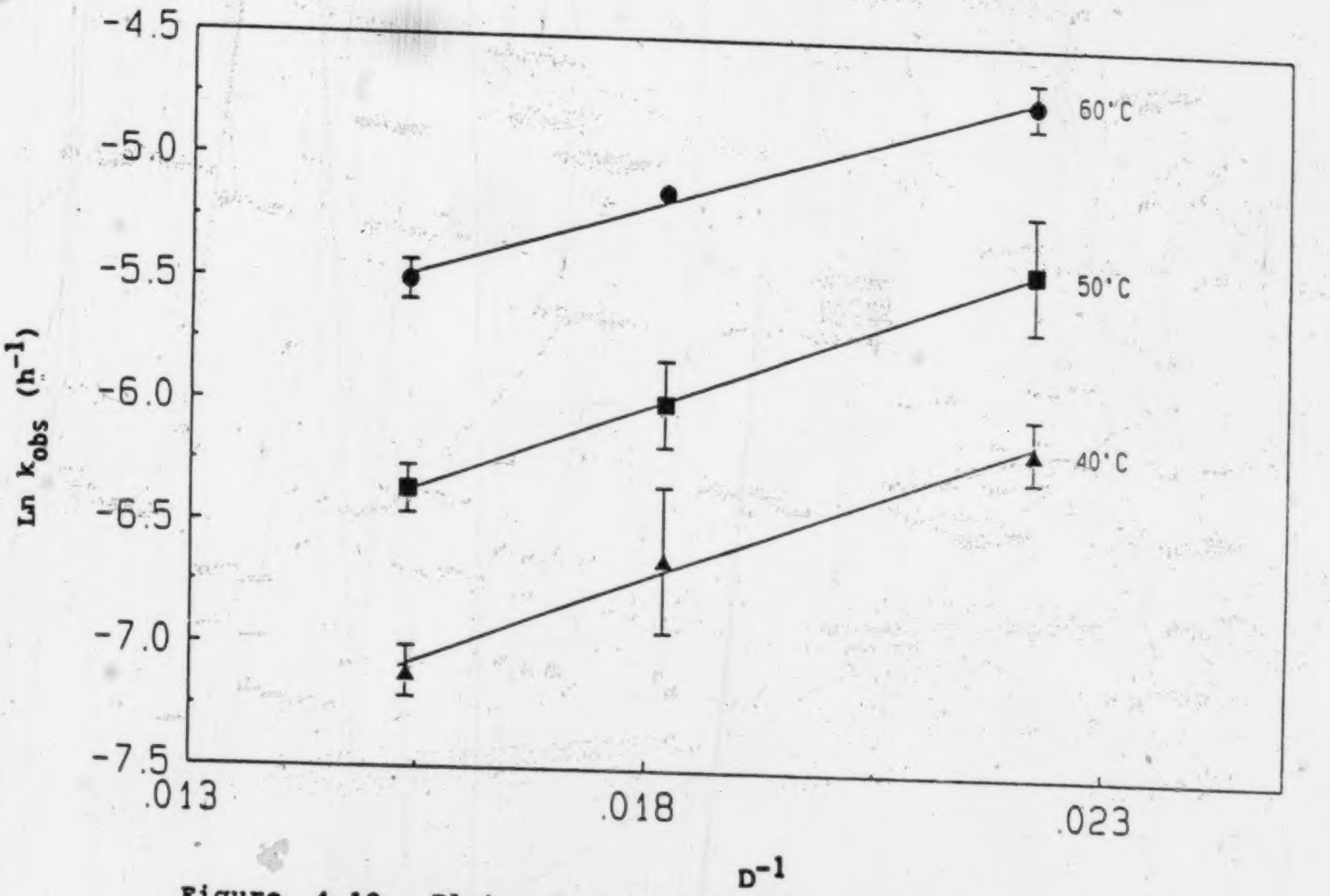


Figure 4.18. Plot of the Observed Rate Constant of Aspartame Degradation Versus the Reciprocal of Dielectric Constant of the Methanol-Water Solvent Mixture. The Dielectric Constant Values of the Solvent Mixtures were 45, 55, and 65.

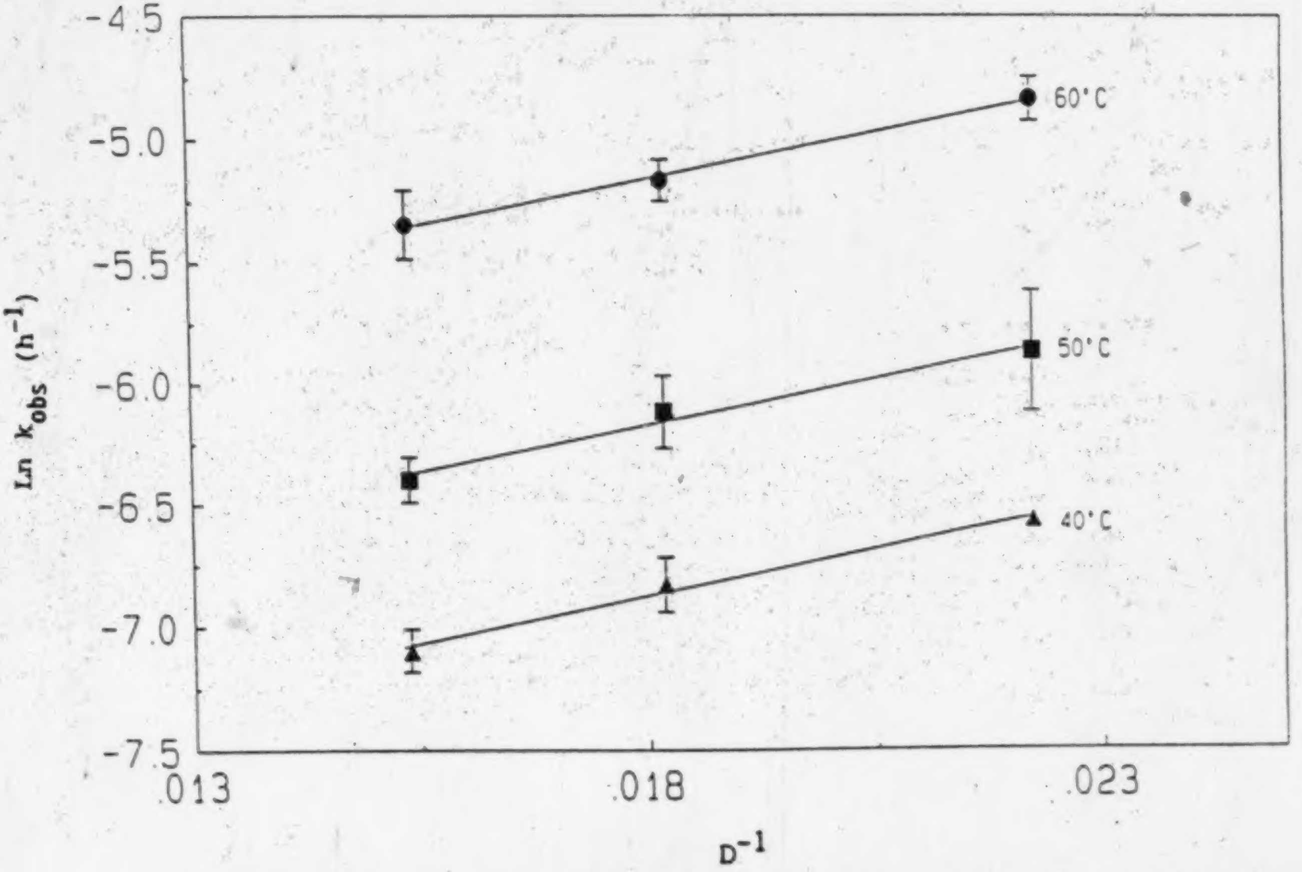


Figure 4.19. Plot of the Observed Rate Constant of Aspartame Degradation Versus the Reciprocal of Dielectric Constant of the Glycerol-Water Solvent Mixture. The Dielectric Constant Values of the Solvent Mixtures were 45, 55, and 65.

and have positive slope. This relationship is expressed by the following equation (24):

$$\ln k_o = \ln k_i - \frac{z_a z_b e^2}{DkTr} \qquad (42)$$

where k_o is a reaction rate at zero ionic strength, k_i is the limiting rate at infinite dielectric constant and zero ionic strength, z_a and z_b are valencies of the two ions, and D, k, T, e, and r are respectively dielectric constant, Boltzmann constant, absolute temperature, electrical charge on ion, and distance to which two ions must approach in order to react. According to equation (42), reaction rate between like-sign ions decreases as dielectric constant decreases, and reaction rate between unlike-sign ions increases as dielectric constant decreases. From Table 4.6 it can be seen that at all three temperatures (40°, 50°, and 60°C) the rate constant of aspartame decomposition decreased as the dielectric constant increased. These results suggest that the observed reaction rate is significantly influenced by the reaction between ions of unlike-signs such as the reaction between the ammonium ion of aspartame and the hydroxide ion. The slope, S, of the plot of natural logarithm of the rate constant of the degradation of aspartame, and the reciprocal of the dielectric constant of the solvent mixtures can be used to calculate r in the following equation (24):

$$S = \frac{d \ln k_o}{d(1/D)} = -\frac{z_a z_b}{kT} \frac{1}{r} \qquad (43)$$

The order of magnitude of r should be that of the radius of a molecule, i.e. 10^{-8} cm. The average values of r, calculated from the slopes of Figs. 4.14 to 4.16, were 4.32×10^{-8} cm, 4.05×10^{-8} cm, and 6.29×10^{-8} cm in water-ethanol, water-methanol, and water-glycerol mixtures respectively. These values are in the range of the theoretical requirement, which is 10^{-8} cm. Another test with respect to the effect of dielectric constant is the difference in activation energy observed when solvents of different dielectric constants are used. For a reaction between like-sign ions, a larger activation energy is necessary in a medium of lower dielectric constant, because repulsion between ions increases as the dielectric constant decreases. The difference in activation energy, dE_c, at dielectric constant D_1 and D_2 is represented as follows (24):

$$dE_c = -329.7 \frac{z_a z_b}{D_1 D_2 r} dD \qquad (44)$$

where dD is the difference of two dielectric constants. Equation (13) demonstrates that the difference in activation energy of the reaction between like-sign ions must increase as the dielectric constant decreases. The activation energies calculated from the Arrhenius plots are shown in Table 4.7.

Table 4.7

Activation Energy of the Decomposition of Aspartame in Water-Alcohol Solutions

Solvent System	Dielectric Constant	Activation Energy (kcal/mole)
	65	17.412
Water-Ethanol	55	15.542
	45	14.664
	65	16.796
Water-Methanol	55	15.501
	45	15.133
	65	19.514
Water-Glycerol	55	18.053
	45	18.032

It is observed that activation energy as well as dE_c increases as dielectric constant of the medium increases. This observation also supports the suggestion that the reaction studied was between unlike-sign ions.

The monohydric alcohols, methanol and ethanol, were used in order to examine whether the molecular size of the alcohol had an effect on the rate of APM degradation. The results showed that the molecular size of the alcohol did not have an effect on the rate of degradation of APM. The rate constants of degradation of APM in methanol-water mixtures were the same, within experimental error, as the rate constants in ethanol-water mixtures (refer to Table 4.6). Methanol was used only for this experimental purpose; it has no practical application as a pharmaceutical solvent because of its toxicity. A more useful pharmaceutical solvent to include in this study would have been propylene glycol; however, information needed to calculate the dielectric constants of mixtures of propylene glycol with water was not available. Dielectric constants of solvent systems used in this study were calculated from tables prepared by Akerlof (146).

Figure 4.20 shows the pH-rate profiles of aspartame using water, 24.7% ethanol-water, and 45.9% ethanol-water as solvents (samples were stored at 60°C). It can be seen that the increase in the degradation rate constant, in the pH range of 3.5 to 4.8, which accompanied the addition of ethanol to the aqueous solution of aspartame, was not caused by a pH

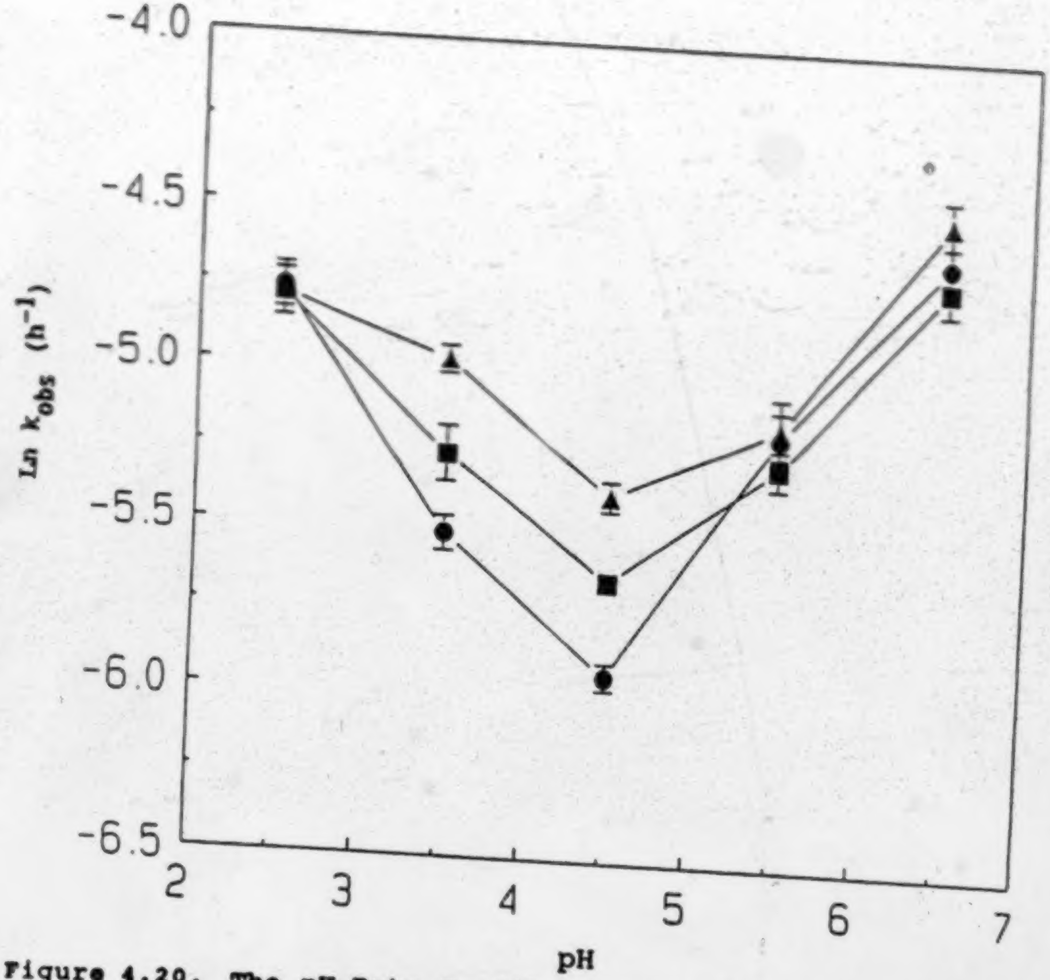


Figure 4.20. The pH-Rate Profile for Aspartame Degradation at 60°C in 45.9% Ethanol-Water Solvent Mixture (▲), 24.7% Ethanol-Water Solvent Mixture (■), and Water (●).

change. The pH-rate plots of aspartame in water-ethanol mixtures were not significantly shifted, either to the right or to the left of the pH-rate plot of aspartame in water. In addition the shapes of these three plots are similar. Therefore, it can be concluded that the increase in the rate of aspartame decomposition which accompanies the addition of ethanol, methanol, or glycerol to aqueous solutions of aspartame may be attributed to a decrease in the dielectric constant of the medium. The stability of aspartame in aqueous solution cannot be enhanced by the replacement of water by solvents of lower dielectric constants.

The pH-rate profile for APM degradation at 60°C in water, Fig. 4.20, shows slopes approaching -1 and +1 (143) at pH values lower than 3.0 and at pH values higher than 5.5, respectively (see page 85). The pH-rate profile, in Fig. 4.20, also showed that the minimum degradation rate constant of APM in malic acid buffer, was on the acid side of the neutral point. Minimum degradation rate constant was at pH 4.5, which means that k_{OH} is larger than k_H (see page 86).

4.4 COMPATIBILITY OF INGREDIENTS IN ACETAMINOPHEN SOLUTION DOSAGE FORM

The UV/visible spectra of aspartame, acetaminophen, methylparaben, and propylparaben were obtained. Also difference UV spectra of binary combinations of the ingredients in the acetaminophen solution dosage form were obtained. These spectra were examined for evidence of formation of stable complexes. Table 4.8 summarizes the observations and comments on these spectra. From these results, it can be stated that this method did not provide any evidence to indicate the formation of stable complexes in the solutions of ingredients of the acetaminophen solution dosage form.

Table 4.8

Results of the Difference Ultraviolet Spectra of the Ingredients of Acetaminophen Solution Dosage Form

Solution	Blank	Comment on Spectrum
Acetaminophen & Aspartame	Acetaminophen	same as spectrum of Aspartame
Acetaminophen & Methylparaben	Acetaminophen	same as spectrum of Methylparaben
Acetaminophen & Propylparaben	Acetaminophen	same as spectrum of Propylparaben
Aspartame & Methylparaben	Aspartame	same as spectrum of Methylparaben
Aspartame & Propylparaben	Aspartame	same as spectrum of Propylparaben

4.5 EVALUATION OF THE ACETAMINOPHEN SOLUTION DOSAGE FORM

The chemical stability of aspartame was evaluated at elevated temperatures. The rate constants of degradation of APM in the acetaminophen solution dosage form are listed in Table 4.9. Table 4.10 presents the statistical analysis of the factorial part of the experiment, that is, formulation number 1 to number 16. An adequate model of the degradation rate constant of APM was found in these experiments:

$$Y = 4.23 + 1.40x_1 + 0.29x_2 + 0.32x_1x_2 + 0.36x_3 + 1.91x_4 + 0.48x_1x_4 + 0.34x_2x_4 + 0.22x_3x_4 + e \tag{45}$$

where Y is the rate constant of APM decomposition and the other symbols are as described in equation 8.

The statistical analysis of the rate constants of APM degradation in the acetaminophen dosage form showed that all four factors studied significantly increased the degradation rate. It is shown in Table 4.10 that when the temperature was increased from 50°C to 60°C, the rate constant increased by a factor of 3.81. Temperature caused the largest increase in the rate constant followed by the effect of pH. When pH was increased from 4.2 to 5.0, the rate constant of APM degradation increased by a factor of 2.80. The factors by which the rate constant of APM degradation increased, when the percent organic solvent was increased from 32% to 40% and when buffer concentration increased from 0.05 M to 0.15 M, were 0.71 and 0.58 respectively.

Data of formulations 17 to 25 were not analyzed statistically because the axial formulations 18 to 25 are not orthogonal to each other and are also not orthogonal to the factorial formulations. However the inclusion of these formulations examining each factor at only two levels increased the scope of the experiment. Instead of examining each factor at only two levels, as done in the factorial part of the experiment, these formulations enable each factor to be studied at five levels. Data from these formulations are rearranged in Tables 4.11 to 4.14.

Data in Tables 4.11 to 4.14 illustrate how the rate constant changes with changes in one factor when all other factors are held constant. Table 4.11 lists data that indicate the rate constant of APM degradation is minimum at pH 4.6, and that it increases as pH is varied in either direction. The rate constant increases faster when pH is raised than when it is lowered from 4.6, thus APM is more stable in acidic than in neutral or alkaline solutions. Tables 4.11 to 4.14 also confirm the results of the statistical analysis of the factorial part of the experiment. These tables show that the largest increase in rate constant

Table 4.9

Result of Aspartame Degradation for a Modified 2^4 Factorial Central Composite Experimental Design

Formulations		Factor Levels in coded form				Rate constant $k\times10^{-3}$/hr	
No.	Type	x_1	x_2	x_3	x_4	Run 1	Run 2
1	factorial	-1	-1	-1	-1	1.21	1.30
2	factorial	+1	-1	-1	-1	2.65	2.49
3	factorial	-1	+1	-1	-1	1.20	1.18
4	factorial	+1	+1	-1	-1	3.61	3.18
5	factorial	-1	-1	+1	-1	1.57	1.62
6	factorial	+1	-1	+1	-1	3.46	3.51
7	factorial	-1	+1	+1	-1	1.57	1.71
8	factorial	+1	+1	+1	-1	3.61	3.68
9	factorial	-1	-1	-1	+1	3.48	3.75
10	factorial	+1	-1	-1	+1	6.75	6.78
11	factorial	-1	+1	-1	+1	4.32	4.03
12	factorial	+1	+1	-1	+1	8.29	8.15
13	factorial	-1	-1	+1	+1	4.48	4.39
14	factorial	+1	-1	+1	+1	8.11	7.89
15	factorial	-1	+1	+1	+1	4.85	4.97
16	factorial	+1	+1	+1	+1	9.13	9.19
17	central	0	0	0	0	6.09	5.73
18	axial	-a	0	0	0	6.87	6.91
19	axial	+b	0	0	0	14.55	15.03
20	axial	0	-a	0	0	5.02	4.78
21	axial	0	+b	0	0	8.65	9.52
22	axial	0	0	-a	0	5.34	5.23
23	axial	0	0	+b	0	5.85	6.61
24	axial	0	0	0	-a	0.66	0.76
25	axial	0	0	0	+b	46.75	44.86

Table 4.10

Analysis of Variance for the 2^4 Factorial Part of the Experiment. The Effect of pH, Buffer Concentration, Solvent Composition, and Temperature on the Degradation of Aspartame in Acetaminophen Solution Dosage Form

Factor	Source	d.f.	Main Effect	Mean Square	F[a]
A	pH	1	2.80	62.86	247.28*
B	Buffer Conc. (M)	1	0.58	2.71	10.69*
C	Solvent Composition	1	0.71	4.04	15.91*
D	Temperature (°C)	1	3.81	116.32	457.95*
AD	pH x Temperature	1	0.95	7.21	28.37*
Error[b]		10		0.254	

[a] Significance level based on 1 d.f. *$p<0.05$.

[b] Error is based on pooling the sum of squares of some 2-factor interactions, all 3-factor interactions and the 4-factor interaction.

Table 4.11

The Effect of pH on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form.

Formulation No.	pH	Average Rate constant (k x $10^{-3}h^{-1)}$
18	3.0	6.89 ± 0.03
17	4.6	5.91 ± 0.25
19	7.0	14.79 ± 0.34

Table 4.12

The Effect of Buffer Concentration on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form.

Formulation No.	Buffer Conc. (M)	Average Rate Constant (k x $10^{-3}h^{-1}$)
20	0.04	4.99 ± 0.30
17	0.10	5.91 ± 0.25
21	0.21	9.09 ± 0.62

Table 4.13

The Effect of Solvent Composition on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form.

Formulation No.	% Organic Solvent	Average Rate Constant ($k \times 10^{-3} h^{-1}$)
22	23	5.29 ± 0.08
17	36	5.91 ± 0.25
23	57	6.23 ± 0.54

Table 4.14

The Effect of Temperature on the Degradation Rate of Aspartame in the Central and Axial Formulations of Acetaminophen Solution Dosage Form.

Formulation No.	Temperature (°C)	Average Rate Constant ($k \times 10^{-3} h^{-1}$)
24	35	0.71 ± 0.07
17	55	5.91 ± 0.25
25	85	45.81 ± 1.34

is brought about by temperature effect, followed by the pH effect. The increases of rate constant brought about by the solvent effect and the buffer concentration were modest.

The rate constants of acetaminophen degradation in the acetaminophen solution dosage form, are presented in Table 4.15 and also the statistical analysis of the factorial part of this experiment is listed in Table 4.16. From these results it can be seen that acetaminophen is more stable than APM by a factor of ten. The statistical analysis of the rate constants of acetaminophen degradation in these formulations showed that pH, solvent composition, temperature and the interaction of solvent composition and temperature were significant. When pH was increased from 4.2 to 5.0, the rate constant of APM degradation decreased, the main effect of pH is -1.56 (see Table 4.16). When both temperature and organic solvent concentration were increased, the rate constant also increased.

4.6 EVALUATIOM OF THE DIAZEPAM SUSPENSION DOSAGE FORM.

Studies on the chemical stability of aspartame and diazepam in the suspension dosage form were carried out at 5°C and at 35°C. Table 4.17 lists the average rate constants of the degradation of APM in the diazepam suspension. Table 4.18 presents the concentration of diazepam remaining in the suspension dosage form with time. The half-lives of APM at 5°C and 35°C were calculated to be 361 and 85 days respectively. This indicated that APM is sufficiently stable

Table 4.15

Result of Acetaminophen Degradation for a Modified 2^4 Factorial Central Composite Experimental Design

Formulations		Factor Levels in coded form				Rate constant $kx10^{-4}$/hr	
No.	Type	x_1	x_2	x_3	x_4	Run 1	Run 2
1	factorial	-1	-1	-1	-1	4.9	4.7
2	factorial	+1	-1	-1	-1	4.7	4.6
3	factorial	-1	+1	-1	-1	7.7	6.9
4	factorial	+1	+1	-1	-1	5.1	5.9
5	factorial	-1	-1	+1	-1	8.8	7.6
6	factorial	+1	-1	+1	-1	4.3	3.7
7	factorial	-1	+1	+1	-1	7.0	6.6
8	factorial	+1	+1	+1	-1	4.4	4.9
9	factorial	-1	-1	-1	+1	7.1	7.4
10	factorial	+1	-1	-1	+1	6.1	5.9
11	factorial	-1	+1	-1	+1	7.0	6.2
12	factorial	+1	+1	-1	+1	7.5	7.4
13	factorial	-1	-1	+1	+1	0.8	11.1
14	factorial	+1	-1	+1	+1	9.9	8.4
15	factorial	-1	+1	+1	+1	9.7	8.5
16	factorial	+1	+1	+1	+1	7.1	6.5
17	central	0	0	0	0	7.2	6.6
18	axial	-a	0	0	0	7.6	7.7
19	axial	+b	0	0	0	8.8	9.4
20	axial	0	-a	0	0	5.0	5.5
21	axial	0	+b	0	0	6.5	7.7
22	axial	0	0	-a	0	9.1	9.2
23	axial	0	0	+b	0	5.3	6.1
24	axial	0	0	0	-a	3.1	3.6
25	axial	0	0	0	+b	53.4	50.8

Table 4.16

Analysis of Variance for the 2^4 Factorial Part of the Experiment. The Effect of pH, Buffer Concentration, Solvent Composition, and Temperature on the Degradation of Acetaminophen in Acetaminophen Solution Dosage Form

Factor	Source	d.f.	Main Effect	Mean Square	F[a]
A	pH	1	-1.56	19.38	17.52*
B	Buffer Conc. (M)	1	-0.06	0.03	0.03
C	Solvent Composition	1	1.32	13.91	12.57*
D	Temperature (°C)	1	2.22	39.38	35.61*
CD	Solvent Comp. x Temp.	1	0.95	7.21	28.37*
Error[b]		5		1.106	

[a] Significance level based on 1 d.f. *$p<0.05$.

[b] Error is based on pooling the sum of squares of all 3-factor interactions and the 4-factor interaction.

in diazepam suspension for it to be used as a sweetener in this dosage form.

The concentration of diazepam in suspensions stored at 5°C and 35°C was virtually constant throughout the two-month study period. Since the concentration of diazepam was equal to or greater than 90 percent of the initial concentration, the results in Table 4.18 show that the diazepam suspension dosage forms, stored at these two temperatures, were stable for at least two months. These suspension dosage forms were also observed visually for changes in color and microbial growth on each sampling day. For the 60-day study period, no detectable changes were seen. All sample maintained their homogeneity and no sign of caking or settling was obversed.

The flow characteristics of the suspension was also evaluated. Figure 4.21 and 4.22 show rheograms of the diazepam suspension before and after shaking by hand respectively. These rheograms were obtained from one sample.

The rheograms in Figs. 4.21 and 4.22 demonstrate that the suspension has a thixotropic flow. Thixotropic flow is defined as a reversible, time-dependent, isothermal gel-sol transition (147). In Fig. 4.21, the stress corresponding to "A" is called the overshoot stress which explains why the formulation is a stable gel when allowed to sit. The stress at "C" is called the yield point which offers resistance to particle-settling. At the point labeled "B" the suspension appears to have broken down to the sol. The down-curve is displaced to the right of the up-curve because the viscosity of the suspension, during the down-curve, is lower at any one point of shear stress. This demonstrates a breakdown of the structure that does not reform immediately when the stress is removed. Rheologic characteristics of a thixotropic system are not unique but will depend on the rhelogic history of the sample and the approach used to obtain the rheogram. This is why the rheograms of the suspension before and after shaking the suspension are different.

In both the acetaminophen solution dosage form and the diazepam suspension dosage form the combination of methyl- and propylparabens is used as the preservative system (see Tables 3.4 and 3.7). In the literature these parabens are often used in the ratio of 10 to 1, methylparaben to propylparaben. It is assumed that these parabens are synergistic, but it has been shown by a number of researchers that preservatives with the same mechanism of action, such as these parabens, may show additive but not synergistic effect (148).

Table 4.17

Results of Aspartame Degradation in Diazepam Suspension

Average Rate Constant ($k \times 10^{-4}$ h^{-1}) for Samples at	
5°C	35°C
0.8 (0.04)[a]	3.4 (0.51)

[a] Standard deviation, n = 4.

Table 4.18

Concentration of Diazepam in Suspensions Stored at 5°C and 35°C

Time	Diazepam Concentration (mg/ml) at	
(day)	5°C	35°C
0	1.18[a] (0.07)[b]	1.15 (0.05)
3	1.12 (0.03)	1.11 (0.04)
9	1.15 (0.04)	1.08 (0.02)
24	1.16 (0.08)	1.06 (0.03)
44	1.13 (0.04)	1.02 (0.05)
60	1.14 (0.03)	1.02 (0.04)

[a] Mean, n = 4.

[b] Standard deviation.

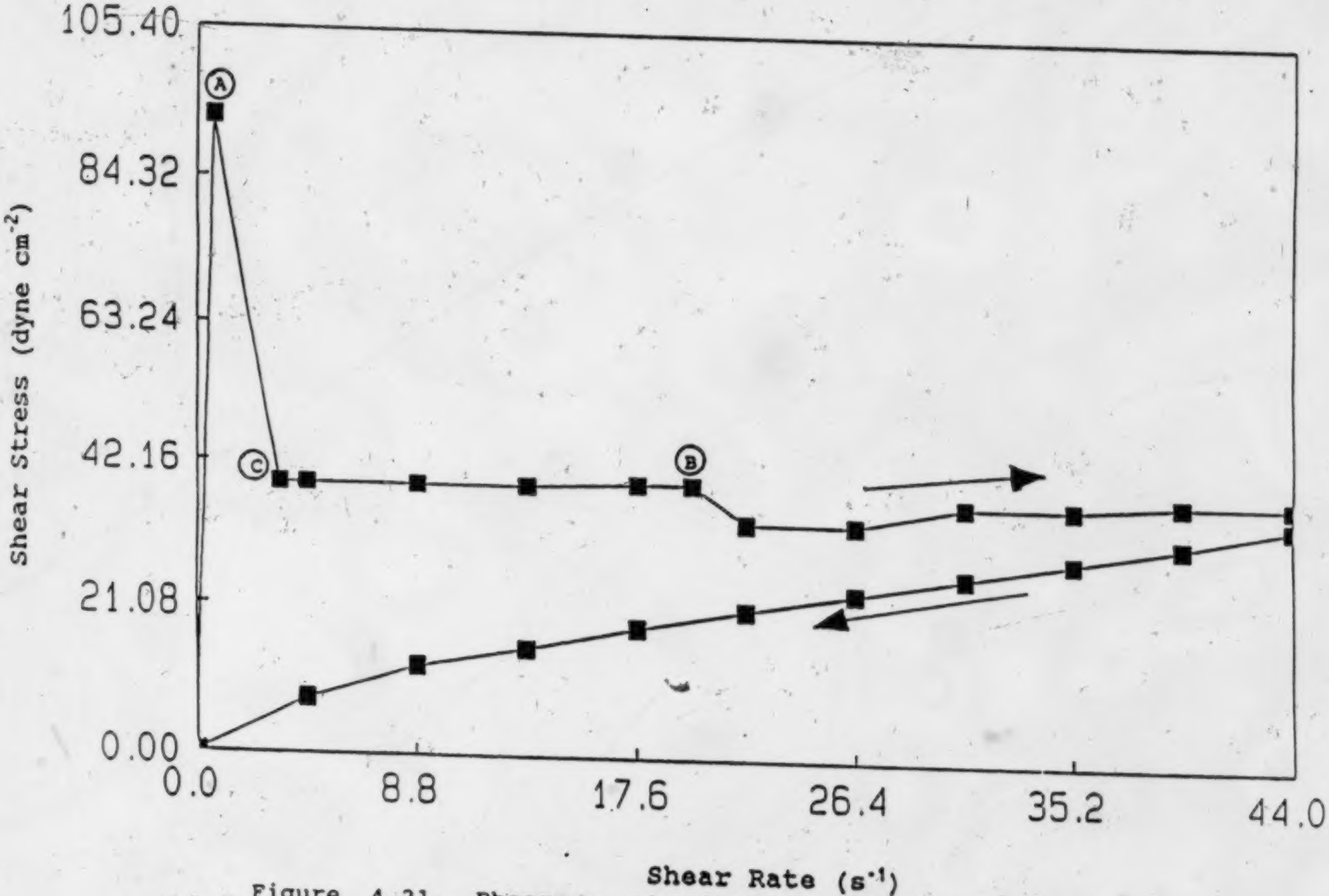


Figure 4.21. Rheogram of Diazepam Suspension, Before Shaking, Showing Thixotropy. The Stress at "A" is Called Overshoot Stress and at "C" is Called Yield Point, Point "B" is where the Gel Appears to Break Down to the Sol.

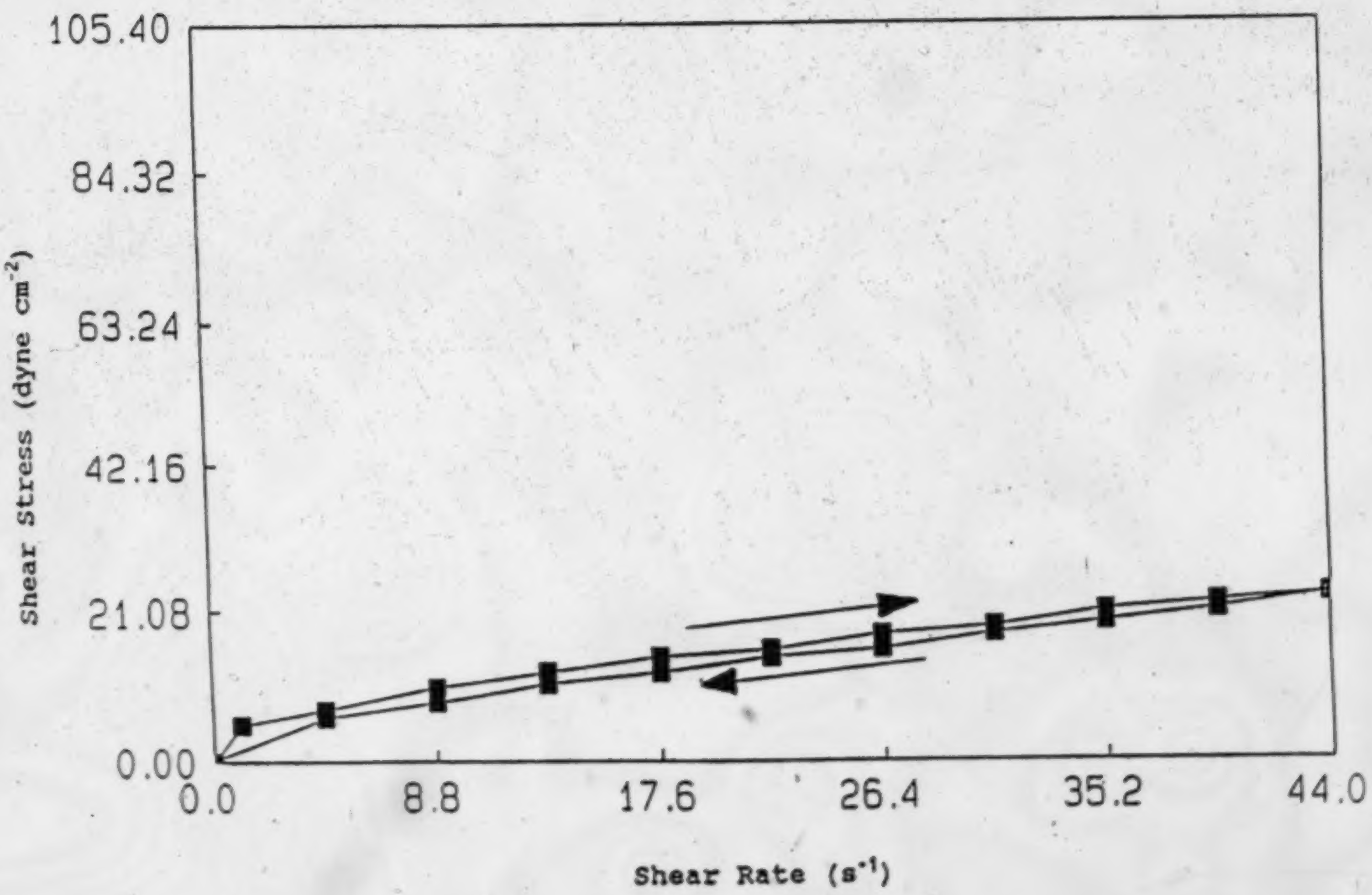


Figure 4.22. Rheogram of Diazepam Suspension, After Shaking, Showing Thixotropy. The Sample Used to Obtain this Rheogram was the Same as Used for Rheogram in Figure 4.22.

4.7 Shelf Life

In this thesis the shelf life of APM is defined as the time period within which APM is able to fulfilling the sweetening requirements of the formulation. APM is a pharmaceutical adjuvant therefore the definition of its shelf life does not have to be the same as the FDA's definition of shelf life for the active ingredients, which was described in the preceding sections (see page 18. It was determined that the minimum concentration of APM required to give satisfactory taste in liquid formulations of acetaminophen and diazepam, was 1 mg/mL. This determination was made by two individuals after tasting a number of different liquid formulations of acetaminophen and diazepam[19]. It was also observed that the degradation products did not cause any discoloration of these liquid dosage forms. Furthermore, it is known that the degradation products of APM do not contribute to any off-flavors and that they are nontoxic. Therefore 1 mg/mL was taken as the minimum acceptable potency level of APM in these liquid dosage forms. Hence the shelf life of APM formulations is the time needed to degrade APM to a minimum concentration of 1 mg/mL.

Given this definition of shelf life of APM and the fact that degradation of APM is apparent first order kinetics, the shelf life of APM depends on the initial concentration of APM in the formulation. In most of our formulations the initial concentration of APM was 5 mg/mL, it is clear that it would take slightly more than two half lives to degrade 5 mg/mL to 1 mg/mL. In our preliminary studies we were able to use 10 mg/mL of APM in the liquid formulations of acetaminophen and diazepam. In these formulations, the shelf life of APM would be greater than three half lives of APM. The calculated $t_{½}$ of APM in the buffered aqueous solutions at 22°C is 291.3 days. It can be shown by simple calculations that if the initial concentration of APM is 10 mg/mL then the shelf life of APM would be 968 days. The $t_{½}$ of APM in diazepam suspension at 5°C is 361 days, given an initial APM concentration of 10 mg/mL, the shelf life of APM would be 3.39 years.

---

[19] Formulations were tasted by Dr.R.A. Locock and S. Sanyude.

5. SUMMARY and CONCLUSIONS

APM has been excluded from liquid pharmaceutical systems because it is known to undergo hydrolysis in these solutions. Stabilization of APM in aqueous and pharmaceutical solvents would increase the use of APM as a sweetener in pharmaceuticals. Therefore, this investigation was carried out in an effort to try to increase the stability of APM in solutions.

Factorial experimental designs were used in the accelerated kinetic studies of APM in buffered aqueous solutions, in mixed solvent systems, and in the acetaminophen solution dosage form. These experimental designs allowed the concurrent examination of many variables on the stability of APM.

The factors which were examined were pH, buffer concentration, buffer species, ionic strength, solvent composition, and temperature. In addition to examining the stability of APM in solution and the suspension dosage forms organoleptic and rheologic characteristics were also evaluated. From these studies the following conclusions can be drawn:

1. The effect of pH on the degradation of APM is significant. The pH-rate profile showed that APM is subject to acid- and base-catalyzed hydrolysis and

that the pH of maximum stability is 4.5, therefore, the stability of APM can be increased substantially if the pH of the system can be adjusted to about this pH.

2. The effect of increasing buffer concentration was to increase the rate constant of APM degradation. This effect was statistically significant. When APM is used in buffered solutions, minimum buffer concentration should be used.
3. Ionic strength did not affect the rate of APM degradation.
4. Buffer species influenced the stability of APM. In the four buffer systems examined, APM exhibited least degradation in malic acid buffer and tartaric acid buffer.
5. Solvent composition significantly affected the stability of APM. The rate constant of APM degradation increased in solvent mixtures with lower dielectric constants. APM cannot be stabilized by partial or total replacement of water by solvents of lower dielectric constants such as ethanol, propylene glycol or glycerol.
6. Temperature has a significant effect on the stability of APM in solution. The rate of APM degradation increases with increase in temperature. Storage of APM solution in cool or refrigerated

temperatures enhances the shelf-life of APM.

7. In the pH range of 3.0 to 6.0 the major route of APM degradation is the cyclization of APM to diketopiperazine.

8. APM can be used as a sweetener in acetaminophen solution dosage forms. With proper attention paid to the previously mentioned factors, the shelf-life APM in this dosage form, can be increased to at least two years.

9. APM can be used to sweeten diazepam suspension; it improved the taste and did not adversely affect the physical s stability of the suspension.

10. The above dosage forms had satisfactory organoleptic properties and were chemically, and physically stable. No microbiologic growth was observed visually.

BIBLIOGRAPHY

1. Boylan, J.C. "Liquids." The Theory and Practice of Industrial Pharmacy. 3rd ed. Lachman, L., Lieberman, H.A. and Kaning, J.K., eds. (Philadelphia: Lea and Febiger, 1986), 468.

2. Nash, R.A. "Pharmaceutical Suspension." Pharmaceutical Dosage Forms: Disperse Systems. Vol. 1, Liberman, H.A., Rieger, M.M. and Banker, G.S., eds. (New York: Marcel Dekker, Inc., 1988), 151-198.

3. Hirst, C.A. and Kaye, R.C. "Effect of Pharmaceutical Formulation on Thioridazine Absorption." J. Pharm. Pharmacol., 23 (1971): 246S-247S.

4. Beck, C.I. "Application Potential for Aspartame in Low Calorie and Dietetic Food." Low Calorie and Special Dietary Foods. B.K. Dwivedi ed. (W. Palm Beach CRC Press, 1978), 68-82.

5. Nabors, L.O. and Gelardi, R.C. "Introduction." Alternative Sweeteners. Nabors, L.O. and Gelardi, R.C., eds. (New York: Marcel Dekker, Inc., 1986), 1-12.

6. Sanyude, S. "Alternative Sweeteners to Sucrose." Can. Pharm. J., 123, (1990): 455-460.

7. Bakal, A.I. "Saccharin Functionality and Safety." Food Tech., 41, (1987): 117-118.

8. Cranmer M. Final Report on Saccharin Report to

Commissioner FDA, pp. 0-21, No. 0-41, 43 (June 7 1978).

9. Cyclamate, *Fed Reg*., 35(167):13644 (Aug 27 1970).

10. Aspartame, *Fed. Reg*., 39, 27317-27319. 1974.

11. Holmer, B.E. "Aspartme: Implications for the Food Scientist." *Aspartame: Physiology and Biochemistry.* Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 247-260.

12. Mazur, R.H. "Aspartame - A Sweet Surprise." *J. Toxicol. Environ. Health*. 2 (1976): 243-249.

13. Neirynck, W. and Nollet, L. "Determination of the Stability of Aspartame in Soft Drinks by Reversed-Phase Liquid Chromatography." *Belg. J. Food Chem. Biotech.*, 43 (1988) 83-88.

14. Mazur, R.H. "Aspartic Acid-Based Sweeteners." *Symposium on Sweeteners*. Englett, G. ed. (Westport: AVI Publishing, 1974), Chap. 14.

15. Swintosky, T.V. *et al.* "Suppression of Penicillin G Procaine Solubility in Aqueous Media." *J. Am. Pharm. Assoc.* 45, (1956): 34-37.

16. Higuchi, T. and Lachman, C. "Inhibition of Hydrolysis of Esters in Solution by Formation of Compexes I: Stabilization of Benzocaine with Caffeine." *J. Am. Pharm. Assoc.* 44, (1956): 521-526.

17. Riegelman, S. "The Effect of Surfactants on Drug Stability I." *J. Am. Pharm. Assoc., Sci. Ed.*, 49, (1960): 339-343.

18. Krasowska, H. "The Hydrolysis of Indomethacin in Aqueous Solutions of Polysorbate." Int. J. Pharm., 4, (1979) 89-97.

19. Cipiciani, A. et al. "Micellar Effects of the Basic Hydrolysis of Indomethacin and Related Compounds." J. Pharm. Sci., 74, (1985): 1184-1187.

20. Sheth, B. and Parrott, E. "Hydrolysis of Solubilized Esters." J. Pharm. Sci., 56, (1967): 983-986.

21. Nelson, E. "Physicochemical Factors Influencing the Absorption of Erythromycin and its Esters." Chem. Pharm. Bull., 10, (1962): 1099-1100.

22. Reigelman, S. and Fischer, E.Z. "Stabilization of Epinephrine against Sulfite Attack." J. Pharm. Sci., 51, (1962): 206-210.

23. Schwartz M.A. and Hayton, W.L. "Relative Stability of Hetacillin and Ampicillin in Solution." J. Pharm. Sci., 16, (1972): 906-909.

24. Ikeda, K. "Studies on Decomposition and Stabilization of Drugs in Solution. IV. Effect of Dielectric Constants on the Stabilization of Barbiturate in Alcohol-Water Mixtures." Chem. Pharm. Bull. 8, (1960): 504-509.

25. Devay, A., Kovacs P. and Rac I. "Optimization of Chemical Stability of Diazepam in the Liquid Phase by Means of Factorial Experimental Design." Int. J. Pharm. Tech. & Prod. Mfr., 6, (1985): 5-9.

26. Jue, J. and Huyck, C.L. "Stability of Aspirin Mixtures."

J. Am. Pharm. Assoc., NS3, (1963): 470-474.

27. Jun, H.W., Whiteworth, C.W. and Luzzi, L.A. "Decomosition of Aspirin in Polyethylene Glycols." J. Pharm. Sci., 61, (1972): 1160-1162.

28. Whiteworth, C.W. and Asker, A.F. "Stability of Aspirin in Liquid and Semisolid Bases V: Polyglycerol Esters." J. Pharm. Sci., 64, (1975): 2018-2020.

29. Asker, A.F. and Whiteworth, C.W. "Evaluation of Dimethylpolysiloxane Fluids as Vehicles for Various Pharmaceuticals I: Effects on the Stability and Dissolution of Aspirin." J. Pharm. Sci., 63, (1974): 1630-1631.

30. Mazur, R.H. and Ripper, A. "Peptide-Based Sweeteners." Developments in Sweeteners. Hough, C.A.M., Parker, K.J. and Vlitos, A.J. eds. (London: Applied Science Publishers, 1979), 125-134.

31. Mazur, R.H., Schlatter, J.M. and Goldkamp, A.H. "Structure-Taste Relationship of Some Dipeptides," J. Am. Chem. Soc., 91, (1969): 2684-22691.

32. Cella, J.A. and Schmitt, W.H. "Low Calorie Dry Sweetener Composition." U.S. Patent 3,753,739, to Aberto Culver Co.; Melrose Park, Ill. (August, 21, 1973).

33. Anonymous (1980). Decision of the Public Board of Inquiry on Aspartame, Docket Number 75F-0335, pp. 1-50. FDA Management Branch, Rockville, MD.

34. Aspartame: Commissioner's Final Decision, Fed. Reg., 46:

38284-38308, 1981.

35. Aspartame: Amendment to Food Additive Regulation for Aspartame in Carbonated Beverages, Fed. Reg., 48: 31376-31382, 1983.

36. Aspartame: Food Additive Permitted for Direct Addition to Food for Human Consumption; Aspartame. Fed. Reg., 51, 42999-43001, 1986.

37. Ripper. A., Holmer, B.E. and Miller, G.A. "Aspartame." Alternative Sweeteners. Nabors, L.O. and Gelardi, R.C., eds. (New York: Marcel Dekker, Inc., 1986), 43-60.

38. Ariyoshi, Y. et al. "Method of Producing Alpha-L-Aspartyl-L-Phenylalalnine Alkyl Esters." U.S. Patent 3,833,553, Ajinomoto Co., Inc., Tokyo, Japan, September 3, 1974.

39. Ariyoshi, Y. and Toi, K. "Method of Purifying Alpha-L-Aspartyl-L-Phenylalanine Lower Alkyl Esters of Peptide Impurities." U.S. Patent 3,833,554, Ajinomoto Co., Inc., Tokyo, Japan, September 3, 1974.

40. Bachman, G.L., Ottedahl, M.L. and Vineyard B.D. "Process for the Preparation of Alpha-L-Aspartyl-L-Phenylalanine Alkyl Esters." U.S. Patent 3,933,781, Monsanto Co., St. Louis Mo., January 20, 1976.

41. Ariyoshi, Y. and Sato, N. "The Separation of Alpha- and Beta-L-Aspartyl-L-Phenylalanine Methyl Ester with Aromatic Acids." Bull. Chem. Soc. Jpn., 45, (1972): 942-943.

42. Ariyoshi, Y. et al. "The Synthesis of a Sweet Dipeptide, Alpha-L-Aspartyl-L-Phenylalanine Methyl Ester, Without the Use of Protecting Groups." Bull. Chem. Soc. Jpn., 46, (1973): 1893-1895.

43. Ganong, W.F. "Taste and Smell." Review of Medical Physiology. 10th ed. (Los Altos, California: Lange Medical Publications, 1981), 140-143.

44. Shallenberger, R.S. and Acree, T.E. "Molecular Theory of Sweet Taste." Nature, 261, (1967): 480-482.

45. Keir, L.B. "A Molecular Theory of Sweet Taste." J. Pharm. Sci., 61, (1972): 1394-1397.

46. Shallenberger, R.S. and Lindley, M.G. "A Lipophilic-Lipophobic Attribute and Component in Stereochemistry of Sweetness." Food Chem., 2, (1977): 145-153.

47. Jakinovich, W. Jr., "Comparative Study of Taste Specificity." Biochemistry of Taste and Olfaction. Cagan, R.H. and Kare, M.R. eds. (New York: Academic Press, 1981): 117-138.

48. Fujino, M. et al. "Structure-TAste Relationship of L-Aspartly-Aminomalonic Acid Diesters. Chem. Pharm. Bull., 24, (1976): 2112-2117.

49. Mapelli, C. et al. "Cyclopropane Amino Acid Ester Dipeptide Sweeteners." Int. J. Pept. Protein Res., 30, (1987):498-510.

50. Shinoda, I. and Okai, H. "Sweetness and Bitterness Contributions of Structural Units of Aspartame and Some

Analogues." J. Agric. Food Chem., 33, (1985): 792-795.

51. Nosho, Y. et al. "Molecular Design of Inverted-Aspartame-Type Sweeteners." J. Agric. Food Chem., 38, (1990):1368-1373.

52. Cloninger, M.R. and Baldwin, R.E. "L-Aspartyl-L-Phenylalanine Methyl Ester (Aspartame) as a Sweetener." J. Food. Sci., 39, (1974): 347-349.

53. Cloninger, M.R. and Baldwin, R.E. "Aspartylphenylalanine Methyl Ester: A Low-Calorie Sweetener." Science, 170, (1970): 81-82.

54. Larson-Powers, N. and Pangborn, R.M. "Paired Comparisons and Time-Intensity Measurements of the Sensory Properties of Beverages and Gelatin Containing Sucrose or Synthetic Sweeteners." J. Food. Sci., 43, (1978) 41-46.

55. Larson-Powers, N. and Pangborn, R.M. "Descriptive Analysis of the Sensory Properties of Beverages and Gelatin Containing Sucrose and Synthetic Sweeteners." J. Food. Sci., 43, (1978): 47-51.

56. Mazur, R.H., Schlatter, J.M. and Goldkamp, A.H. "Structure-Taste Relationship of Some Dipepitides." J. Am. Chem. Soc.,91, (1969): 2684-2691.

57. McPherson, B.A.; McGill, L.A. and Bodyfelt, F.W. "Effect of Stabalizing Agents and Aspartame on the Sensory Properties of Orange Serbet." J. Food. Sci., 43, (1978): 935-939.

58. Moskowitz, H.R. and Dubose, C. "Taste Intensity,

Pleasantness and Quality of Aspartame Sugars, and Their Mixtures." Can. Inst. Food. Sci. Technol. J. 10, (1977): 126-131.

59. Porikos, K.P., Booth, G. and Van Itallie, T.B. "Effect of Covert Nutritive Dilution on the Spontaneous Food Intake of Obese Individuals: A Pilot Study." Am. J. Nutr., 30, (1977): 1638-1644.

60. Schiffman, S.S. "Comparison of the Taste of Aspartame with Other Sweeteners." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 207-246.

61. Schiffman, S.S., Cahn, H. and Lindley, M.G. "Multiple Receptor Sites Type Mediate Sweetness: Evidence from Cross Adaption." Pharmacol. Biochem. Behav., 15, (1981): 377-388.

62. Schiffman, S.S., Reilly, A.D. and Clark, 111, T.B. "Qualatative differences among sweeteners." Physiol. Behav., 23, (1979): 1-9.

63. Stone, H. and Oliver, S.M. "Measurement of Relative Sweetness of Selected Sweeteners and Sweeteners Mixtures." J. Food Sci, 34, (1969): 215-222.

64. Inglett, G.E. "Sweeteners: An Overall Perspective." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 11-22.

65. Schade, H.R. "Taste Modifiers for Artificial Sweeteners."

U.S. Patent No. 3,934,047, to General Food Corp., White Plains, N.Y., Jan. 20, 1976.

66. Eisenstadt, M.E. "Calorie-Free Dipeptide Sweetening Composition." U.S. Patent 3,875,311, Cumberland Packing Corporation, Bracebridge, Ont., Canada, April, 1975.

67. Eisenstadt, M.E. "Dipeptide sweetener composition." U.S. Patent 3,875,312, Cumberland Packing Corp, April 1, 1975.

68. Baldwin R.E. and Korschgen, B.M. "Intensification of Fruit-Flavors by Aspartame." J. Food Sci., 44, (1979):938-939.

69. Fung, H. "Chemical Kinetics and Drug Stability." Modern Pharmaceutics, 2nd ed., Banker, G.S. and Rhodes, C.T. eds. (New York: Marcel Dekker, Inc., 1990), 209-237.

70. Lintner, C.J. "Stability of Pharmaceutical Products." Remington's Pharmaceutical Sciences, 17th ed. Gennaro, A.R. ed. (Easton PA: Marck Publishing Company, 1985), 1478-1486.

71. Dearborn, E.H. The Dating of Pharmaceuticals. Windheuser, J.J. and Blockstein eds., (Madison: University Extension, University of Wisconsin, 1970), 29.

72. Anonymous. "Aspartame Safety Issues: A Scientific Update." International Food Information Council, 1250 Eye Street NW, Suite 300, Washington, D.C. 20005, 1986.

73. Pagliaro, L.A. and Locock, R.A. "Aspartame." Can. Pharm. J., (1986): 121-123.

74. Stegink, L.D. "Aspartame: Review of the Safety Issues."

Food Technol., (1987): 119-121.

75. Janssen, P.J.C.M. and der Heijden, C.A. "Aspartame: Review of Recent Experimental and Observational Data." Toxicol., 50, (1988): 1-26.

76. Stegink, L.D. "Aspartame Metabolism in Humans." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 509-553.

77. Abrams, I.J. "Using the Menu Census Survey to Estimate Dietary Intake: Post Marketing Surveillance of Aspartame." International Aspartame Workshop Proceedings (ILSI/NF), November 17-21, 1986, Marbella, Spain.

78. Tephly, T.R. and McMartin, K.E. "Methanol Metabolism and Toxicity." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 111-140.

79. Stegink, L.D. "Aspartate and Glutamate Metabolisms." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 47-76.

80. Pardridge, W.M. "Potential Effects of the Dipeptide Sweetener Aspartame on the Brain." Nutrition and the Brain. Wurtman, R.J. and Wurtman J.J., eds., vol. 7. (New York: Raven Press, 1986), 199-241.

81. Applebaum, A.E., Daabees, T.T. and Stegink, L.D., "Aspartate-Induced Neurotoxicity in Infant Mice."

Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 349-362.

82. Stegink, L.D. et al. "Comparative Metabolism of Glutamate in the Mouse, Monkey and Man." Glutamic Acid Advances in Biochemistry and Physiology. Filer et al., eds. (New York: Raven Press, 1979), 85-102.

83. Stegink, L.D. et al. "Factors Affecting Plasma Glutamate Levels in Normal Adult Subjects." Glutamic Acid Advances in Biochemistry and Physiology. Filer et al., eds. (New York: Raven Press, 1979), 333-351.

84. Stegink, L.D. et al. "Monosodium Glutamate Metabolism in Neonatal Monkey." Am. J. Physiol., 229, (1975): 246-250.

85. Olney, J.W., Sharpe, L.G. and Feigin, F.D. "Glutamate-Induced Brain Damage in Infant Primates." J. Neuropathol. Exp. Neurol., 31, (1972): 464-488.

86. Abraham, R. et al. "The Response of the Hypothalamus to High Doses of Monosodium Glutamate in Mice and Monkeys." Toxicol. Appl. Pharmacol., 19, (1971): 388-389.

87. Abraham, R. et al. "Electron Microscopic Observations of Hypothalami in Neonatal Rhesus Monkeys (Macacca mulatta) after Administration of Monosodium-L- glutamate." Exp. Mol. Pathol., 23, (1975): 203-213.

88. Heywood. R. and Prentice D.E. "The Effect of Oral Administration of Monosodium Glutamate to Premature

Rhesus Monkeys." Huntington Research Center Report No. 36/75994, 1975.

89. Heywood, R. and James, R. W. "An Attempt to Induce Neurotoxicity in an Infant Rhesus Monkey with Monosodium Glutamate." Toxicol. Lett., 4, (1979): 285-286.

90. Reynolds, W. A., Butler, V. and Lemkey-Jonston. "Hypothalamic Morphology After Ingestion of Aspartame or MSG in Neonatal Rodents and Primates: A Preliminary Report." J. Toxicol. Environ. Health, 2, (1976): 471-480.

91. Oser, B.L., Morgareidge, K. and Carson, S. "Monosodium Glutamate Studies in Four Species of Neonatal and Infant Animals." Food Cosmet. Toxicol., 13, (1975): 7-14.

92. Reynolds, W.A., Parsons, L. and Stegink, L.D. "Neuropathology Studies Following Aspartame Ingestion by Infant Non-Human Primates." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 363-378.

93. Garattini, S. "Evaluation of Neurotoxic Effects of Glutamic Acid." Nutrition and the Brain. Wurtman, R.J. and Wurtman J.J., eds., vol. 4. New York: Raven Press, 1979, 79-124.

94. IGTC-Internationl Glutamate Technical Committee, Scientific Literature Review on Glutamates, Report dated September 10, 1985.

95. Okaniwa, A. et al. "Histopathological Study of the

Effects of Potassium Aspartate on the Hypothalamus of Rats." J. Toxicol. Sci., 4, (1979): 31-39.

96. Baker G. L. "Aspartame Ingestion During Lactation." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 565-577.

97. Roak-Foltz, R. and Leveille, G.A. "Projected Aspartame Intake: Daily Ingestion of Aspartic Acid, Phenylalanine, and Methanol." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 201-205.

98. Meyers, F.H., Jawetz, E. and Goldfrien, A. "Adrenergic Drugs." Review of Medical Pharmacology. 7th ed. (Los Altos: Lange Medical Publications, 1980), 86.

99. Harper, A.E. "Phenylalanine metabolism." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 77-104.

100. Krassner, M.B. "Diet and Brain Function." Nutr. Rev., 14, supplement May 1986, 12-15.

101. Fernstrom, J.D. "Effects of Aspartame on Large Neutral Amino Acids and Monoamine Neurotransmitters in Rat Brain." International Workshop Proceedings (ILSI/NF), Marbella, Spain, November 17-21, 1986.

102. Pardridge, W.M. and Olderndorf, W.H. "Transport of Metabolic Substrates Through the Blood Brain Barrier." J. Neurochem., 28, (1977): 5-12.

103. Fernstrom, J.D. "Oral Aspartame and Plasma Phenylalanine: Pharmacokinetic Difference Between Rodents and Man, and Relevance to CNS Effects of Phenylalanine." J. Neural Transm., 75, (1989): 159-164.

104. Kaufman, S. "The Enzymology of Phenylketonuria." International Aspartame Workshop Proceedings (ILSI/NF), Marbella, Spain, November 17-21, 1986.

104. Caballero, B. and Wurtman, R.J. "Control of Phenylalanine Levels, in Dietary Phenylalanine and Brain Function." Wurtman, R.J. and Ritter-Walker, E., eds. Proceedings of the First International Meeting of Dietary Phenylalanine and Brain Function, Washington D.C., May 8-10, pp. 9-15, 1987.

106. Coulombe, R. and Sharma, R.P. "Neurobiochemical Alteration Induced by the Artificial Sweetener Aspartame (Nutrasweet)." Toxicol., Appl. Pharmacol., 83, (1986): 79-85.

107. Wurtman, R.J. "Neurochemical Changes Following High-Dose Aspartame With Dietary Carbohydrates." N. Engl. J. Med., 309 (1983): 429-430.

108. Vaughan, D.A., Womack, M. and McClain. "Plasma Free Amino Acid Levels in Human Subjects After Meals Containing Lactalbumin, Heated Lactalbumin or Protein." Am. J. Clin. Nutr., 30 (1977): 1709-1712.

109. Matalon, R. et al. "Aspartame Consumption in Normal Individuals and Carriers for Phenylketonuria (PKU)."

Dietary Phenylalanine and Brain Function. Wurtman R.J. and Ritter-Walker, E. eds. Proceedings of the First International Meeting of Dietary Phenylalanine and Brain Function, Washington D.C., May 8-10, pp. 81-94, 1987.

110. Filer, L.J. and Stegink, L.D. "Effects of Aspartame on Plasma, Phenylalanine Concentration in Humans. Wurtman, R.J. and Ritter-Walker, E. eds. Dietary Phenylalanine and Brain Function. Proceedings of the First International Meeting of Dietary Phenylalanine and Brain Function, Washington D.C., May 8-10, pp. 25-56, 1987.

111. Visek, W.J. "Chronic Ingestion of Aspartame in Humans." Aspartame: Physiology and Biochemistry. Stegink, L.D. and Filer, L.J. Jr., eds. (New York: Marcel Dekker, 1984), 77-104.

112. Cabellero, B. et al. "Plasma Amino Acid Levels After Single Dose Aspartame Consumption in Phenylketonuria, Mild Hyperphenylalaninemia, and Heterozygous State for Phenylketonuria." J. Pedriatr., 109, (1986): 668-671.

113. Guttler, F. and Lou, H. "Aspartame May Imperil Dietary Control of Phenylalanine." Lancet, 1, (1985): 525-526.

114. Anonymous. "Evaluation of Consumer Complaints Related to Aspartame Use." Division of Nutrition, Center for Health Promotion and Education, Center for Disease Control, Atlanta, Georgia 30333. Report received November 1984.

115. Glinsman, W.H., Tollefson, L.K. and Hattan, D.G. "Evaluation of the Adverse Reactions Reported to be

Associated with the Use of Aspartame-Containing Products." Report for the Center of Food Safety and Applied Nutrition, Food and Drug Administration, Washington. Report undated, estimated publication year: 1987.

116. Bradstock, M.K. _et al._ "Evaluation of Reactions to Food Additive: the Aspartame Experience." _Am. J. Clin. Nutr._, 43, (1986): 464-469.

117. Roberts, H.J. "Neurologic, Psychiatric and Behavioral Reaction to Asaprtame in 505 Aspartame Reactors." Wurtman, R.J. and Ritter-Walker, E. eds. _Dietary Phenylalanine and Brain Function._ Proceedings of the First International Meeting of Dietary Phenylalanine and Brain Function, Washington D.C., May 8-10, pp. 477-481, 1987.

118. Cho, E. S., Coon, J.D. and Stegink, L.D. "Plasma and Urine Diketopiperazine Concentrations in Normal Adults Ingesting Large Quantities of Aspartame." _Fd. Chem. Toxic._ 25, (1986): 499-503.

119. Hattan, D. "Toxicological and Nutritional Evaluation of Low Nutrient Density Foods." _Nutritional and Toxicological Aspects of Food Processing_. ed. Walker, R. and Quattrucci, E. (London: Taylor & Francis Ltd., 1988), 275-289.

120. Koshy, K.T. and Lach, J.L. "Stability of Aqueous Solutions of N-Acetyl-p-aminophenol." _J. Pharm. Sci._,

50, (1961): 113-118.

121. Connors, K.A., Amidon, G.L. and Stella V. S. "Acetaminophen." Chemical Stability of Pharmaceuticals. 2nd. ed. (New York: John Wiley & Sons, 1986), 163-168.

122. Han, W. W., Yakata, G. J. and Maness, D. D. "Kinetics and Mechanisms of Hydrolysis of 1,4-Benzodiazepines II: Oxazepam and Diazepam." J. Pharm. Sci., 66, (1977): 573-577.

123. Nakano, M. et al. "Reversible Ring-Opening Reaction of Diazepam in Acid-Media Around Body-Temperature." Int. J. Pharmaceut., 3, (1979)195-204.

124. Verzella, G. and Mangia, A. "High-Performance Liquid Chromatographic Analysis of Aspartame." J. Chromatogr., 346, (1985): 417-422.

125. Waltersson, J.-O. "Factorial Designs in Pharmaceutical Preformulation Studies." Acta Pharm. Suec. 23, (1986): 129-138.

126. Bolton, S. "Factorial Designs in Pharmaceutical Stability Studies." J. Pharm. Sci., 72, (1983): 362-366.

127. Bolton, S., Reinstein, J. and Alobe, O. "Application of Factorial Designs in Kinetic Studies: Hydrolysis of Benzypenicillin Solutions." Int. J. Pharm. Tech. & Prod. Mfr. 5, (1984): 6-12.

128. Devay, A., Kovacs, P. and Rac, I. "Optimization of Chemical Stability in the Liquid Phase by Means of Factorial Experimental Design." Int. J. Pharm. Tech. &

Prod. Mfr., 6, (1985): 5-9.

129. Davies, O.L. "Factorial Experiments:Elementary Principles." *The Design and Analysis of Industrial Experiments*. (New York: Longman Inc., 1978), Chap. 7.

130. Bolton, S. "Factorial Design." *Pharmaceutical Statistics*, 2nd ed., (New York: Marcel Dekker, Inc., 1984), Chap. 9.

131. Sena, F.J., Piechocki, J.T. and Li, K.L. "Stability-Indicating Assay of Acetaminophen in an Effervescent Tablet by Ion-Pair High-Performance Liquid Chromatography." *J. Pharm. Sci*., 68, (1979):1465-1466.

132. Emery, M. and Kowtko, J. "High-Performance Liquid Chromatography of Benzodiazepines I: Stability-Indicating Assay of Diazepam Tablets." *J. Pharm. Sci*., 68, (1979): 1185-1187.

133. Lachman, L., Deluca, P. and Akers, M.J. "Kinetic Principles and Stability." *The Theory and Practice of Industrial Pharmacy*. 3rd ed. Lachman, L., Lieberman, H.A. and Kaning, J.K., eds. (Philadelphia: Lea and Febiger, 1986), 766.

134. Connors, K.A. "Interpretation of Kinetic Data." *Chemical Stability of Pharmaceuticals.* Connors, K.A., Amidon, G.L. and Stella V. S. eds., 2nd. ed. (New York: John Wiley & Sons, 1986), 163-168.

135. Bell, R.P. "The Classical Theory of Catalysis by Hydrogen and Hydroxyl Ions." *Acid-Base Catalysis*. (Oxford

University Press, Amen House, London E.C.4, 1941), 1-10.

136. Racz, I. "Drug Stability." Drug Formulation. (Budapest, Hungary, 1989), Chap. 2.

137. Martin, A., Swarbrick, J. and Cammarata, A. "Kinetics." Physical Pharmacy, 3rd ed. (Philadelphia: Lea & Febiger 1983), 352-398.

138. Finholt, P., Jurgensen, G. and Kristiansen, H. "Catalytic Effect of Buffers on Degradation of Pencillin G in Aqueous Solution." J. Pharm. Sci., 54, (1965): 387-393.

139. Hou, G.N. and Poole, J.W. "Kinetics and Mechanism of Degradation of Ampicillin in Solution." J. Pharm. Sci., 58, (1969): 447-454.

140. Bell, R.P. "Salt Effect." Acid-Base Catalysis. (Oxford University Press, Amen House, London E.C.4, 1941), 11-35.

141. Lewis, G.N. and Randall, M. "A Usefull Function, Called the Activity and its Application to Solutions." Thermodynamics and The Free Energy of Chemical Substance. (New York: McGraw-Hill, 1923):254-273.

142. Carstensen, J.T. "Kinetic Salt Effect in Pharmaceutical Investigations." J. Pharm. Sci., 59, (1970): 1140-1143.

143. Robert, L.E. and Hem, S.L. "Effects of Ionic Strength on Chemical Stability of Potassium Penicillin G." J. Pharm. Sci., 61, (1972):202-206.

144. Martin, A., Swarbrick, J. and Cammarata, A. "Buffered and Isotonic Solutions." Physical Pharmacy, 3rd ed. (Philadelphia: Lea & Febiger 1983), 222-244.

145. Bates, R.G. "Buffer Solutions." Determination of pH, 2nd ed. (New York: John Wiley & Sons, 1964), 95-130.

146. Akerlof, G. "Dielectric Constants of Some Organic Solvent-Water Mixtures at Various Temperatures." J. Am. Chem. Soc., 54, (1932): 4125-4139.

147. Deem, D.E. "Rheology of Dispersed Systems." Pharmaceutical Dosage Forms: Dispersed Systems. Vol. 1. Lieberman, H.A., Rieger, M.M., and Banker, S.G. eds. (New York: Marcel Dekker Inc., 1988), Chap. 9.

148. Denyer, S.P., Hugo, W.B,, and Harding, V.D. "Synergy in Preservative Combinations." Int. J. Pharmaceut., 25 (1985): 245-253.

APPENDIX

Table A.1

Decoded Values of a Modified 2^4 Factorial Central Composite Experimental Design

Formulations No.	Factors			
	pH	Buffer Conc (M)	%Organic Phase	Temperature (°C)
1	4.2	.05	32	50
2	5.0	.05	32	50
3	4.2	.15	32	50
4	5.0	.15	32	50
5	4.2	.05	40	50
6	5.0	.05	40	50
7	4.2	.15	40	50
8	5.0	.15	40	50
9	4.2	.05	32	60
10	5.0	.05	32	60
11	4.2	.15	32	60
12	5.0	.15	32	60
13	4.2	.05	40	60
14	5.0	.05	40	60
15	4.2	.15	40	60
16	5.0	.15	40	60
17	4.6	.10	36	55
18	3.0	.10	36	55
19	7.0	.10	36	55
20	4.6	.04	36	55
21	4.6	.21	36	55
22	4.6	.10	23	55
23	4.6	.10	57	55
24	4.6	.10	36	35
25	4.6	.10	36	85

Table A.2

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Citric Acid Buffer Solutions at 60°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr)		d.f.	Calculated Effect	Mean Square	F[a]
	Run 1	Run 2				
(1)	5.57	5.76	-	-	-	-
a	9.70	9.30	1	4.53	40.95	559.44*
b	5.45	5.09	1	-0.20	0.08	1.09
ab	9.58	9.74	1	0.11	0.02	0.33
c	7.99	7.26	1	2.47	12.15	166.02*
ac	12.66	12.60	1	0.42	0.34	4.71
bc	7.47	7.35	1	-0.08	0.01	0.17
abc	12.54	12.03	1	-0.17	0.06	0.79
Error			8		0.073	

[a] Significance level based on 1 d.f. *$p<0.05$.

Table A.3

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Citric Acid Buffer Solutions at 50°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr)		d.f.	Calculated Effect	Mean Square	F[a]
	Run 1	Run 2				
(1)	1.81	1.68	-	-	-	-
a	3.63	3.52	1	2.46	12.05	1772.66*
b	1.73	1.67	1	-0.07	0.01	1.24
ab	3.31	3.31	1	-0.05	0.00	0.00
c	2.83	2.81	1	1.84	6.73	990.38*
ac	5.99	5.98	1	0.74	1.08	158.90*
bc	2.78	2.87	1	0.09	0.02	2.66
abc	6.17	5.91	1	0.07	0.01	1.24
Error			8		1.154	

[a] Significance level based on 1 d.f.; *$p<0.05$.

Table A.4

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Citric Acid Buffer Solutions at 40°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	0.85	0.80	-	-	-	-
a	1.59	1.58	1	10.45	218.41	189.30*
b	0.79	0.86	1	0.15	0.05	0.04
ab	1.63	1.40	1	0.53	0.55	0.48
c	1.31	1.32	1	7.73	119.35	103.45*
ac	2.60	2.48	1	3.20	20.48	17.75*
bc	1.32	1.16	1	0.50	0.50	0.43
abc	2.60	2.89	1	0.88	1.53	1.33
Error			8		1.154	

[a] Significance level based on 1 degree of freedom; *$p<0.05$.

Table A.5

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Malic Acid Buffer Solutions at 60°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr)		d.f.	Calculated Effect	Mean Square	F[a]
	Run 1	Run 2				
(1)	3.51	3.11	-	-	-	-
a	6.85	5.86	1	3.28	21.52	27.95*
b	3.43	3.07	1	-0.205	0.08	0.11
ab	6.26	5.43	1	-0.08	0.01	0.02
c	5.45	4.71	1	2.16	9.33	12.12*
ac	9.83	7.67	1	0.46	0.41	0.54
bc	5.14	4.63	1	0.08	0.01	0.02
abc	9.79	7.58	1	0.15	0.04	0.05
Error			8		0.770	

[a] Significance level based on 1 d.f.; *$p<0.05$.

Table A.6

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Malic Acid Buffer Solutions at 50°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	1.36	1.32	-	-	-	-
a	2.32	2.33	1	1.26	3.19	924.00*
b	1.29	1.23	1	-0.10	0.02	6.09
ab	2.23	2.22	1	-0.03	0.00	0.00
c	1.96	1.90	1	0.87	1.52	441.31*
ac	3.46	3.55	1	0.28	0.15	44.64*
bc	1.80	1.92	1	-0.01	0.00	0.00
abc	3.28	3.43	1	-0.01	0.00	0.00
Error			8		0.003	

[a] Significance level based on 1 d.f. *$p<0.05$.

Table A.7

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Malic Acid Buffer Solutions at 40°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	0.63	0.57	-	-	-	-
a	1.15	1.13	1	5.53	61.05	174.43*
b	0.63	0.58	1	-0.20	0.08	0.23
ab	1.04	0.96	1	-0.25	0.13	0.36
c	0.94	0.84	1	3.75	28.13	80.36*
ac	1.58	1.43	1	0.85	1.45	4.13
bc	0.94	0.85	1	0.48	0.45	1.29
abc	1.53	1.58	1	0.48	0.45	1.29
Error			8		0.35	

[a] Significance level based on 1 d.f. *$p<0.05$.

Table A.8

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Tartaric Acid Buffer Solutions at 60°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr)		d.f.	Calculated Effect	Mean Square	F^a
	Run 1	Run 2				
(1)	3.04	2.86	-	-	-	-
a	3.56	3.22	1	0.70	0.97	28.86*
b	2.98	2.95	1	-0.15	0.04	1.29
ab	3.45	2.95	1	-0.12	0.03	0.82
c	4.29	4.11	1	1.56	4.88	144.84*
ac	5.45	5.32	1	0.36	0.26	7.80*
bc	4.27	3.97	1	-0.06	0.01	0.23
abc	5.06	5.03	1	-0.01	0.00	0.01
Error			8		0.034	

[a] Significance level based on 1 d.f. * $p<0.05$.

Table A.9

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Tartaric Acid Buffer Solutions at 50°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Rate constant ($k \times 10^{-3}$/hr) Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	1.24	1.27	-	-	-	-
a	1.41	1.50	1	0.42	0.34	12.84*
b	1.13	1.[illegible]5	1	0.06	0.01	0.27
ab	1.37	1.40	1	0.09	0.01	0.54
c	1.67	1.74	1	0.71	1.01	37.58*
ac	2.03	2.30	1	0.22	0.09	3.45
bc	1.71	1.75	1	0.13	0.03	1.26
abc	2.81	2.24	1	0.09	0.01	0.54
Error			8		0.03	

[a] Significance level based on 1 d.f. * $p<0.05$.

Table A.10

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Tartaric Acid Buffer Solutions at 40°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	0.67	0.99	-	-	-	-
a	0.85	0.88	1	1.98	7.80	7.65*
b	0.67	0.71	1	-0.98	1.90	1.86
ab	0.72	0.86	1	-0.88	1.53	1.50
c	0.92	0.85	1	3.18	20.16	19.77*
ac	1.42	1.41	1	1.28	3.25	3.19
bc	0.92	1.10	1	0.13	0.03	0.03
abc	1.11	1.14	1	-1.18	2.76	2.71
Error			8		1.02	

[a] Significance level based on 1 d.f. *$p<0.05$.

Table A.11

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Citric Acid Buffer Solutions with 0.05% EDTA at 60°C.

Source of Variation	Rate constant (k x 10^{-3}/hr)		d.f.	Calculated Effect	Mean Square	F^a
	Run 1	Run 2				
(1)	7.07	7.75	-	-	-	-
a	14.60	14.10	1	9.81	192.57	554.70^*
b	7.33	7.74	1	0.32	0.18	0.53
ab	15.14	13.97	1	0.32	0.21	0.60
c	10.67	10.21	1	5.72	65.38	188.33^*
ac	23.12	21.85	1	2.84	16.10	46.38^*
bc	10.70	9.86	1	0.14	0.04	0.11
abc	23.97	23.09	1	0.28	0.15	0.44
Error			8		0.347	

[a] Significance level based on 1 d.f. $^*p<0.05$.

Table A.12

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Malic Acid Buffer Solutions with 0.05% EDTA at 60°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr)		d.f.	Calculated Effect	Mean Square	F^a
	Run 1	Run 2				
(1)	4.10	3.78	-	-	-	-
a	6.85	6.71	1	3.84	29.53	1824.2^*
b	3.72	3.54	1	0.07	0.01	0.56
ab	6.86	6.81	1	0.20	0.08	5.07
c	5.45	5.43	1	2.49	12.43	767.57^*
ac	9.86	9.90	1	0.82	1.34	82.57^*
bc	5.53	5.43	1	0.19	0.07	4.58
abc	10.51	10.21	1	0.02	0.00	0.04
Error			8		0.016	

[a] Significance level based on 1 d.f. $^*p<0.05$.

Table A.13

Results and Analysis of Variance for a 2^3 Factorial Experiment (Run in Duplicate). The Effect of pH, Buffer Concentration and Ionic Strength on the Degradation of Aspartame in Tartaric Acid Buffer Solutions with 0.05% EDTA at 60°C.

Source of Variation	Rate constant ($k \times 10^{-3}$/hr) Run 1	Rate constant ($k \times 10^{-3}$/hr) Run 2	d.f.	Calculated Effect	Mean Square	F[a]
(1)	3.75	4.16	-	-	-	-
a	4.36	5.14	1	1.32	3.46	12.28*
b	4.06	3.70	1	-0.38	0.29	1.06
ab	4.52	4.65	1	0.23	0.10	0.36
c	6.42	8.04	1	3.31	21.91	77.81*
ac	8.17	9.07	1	0.57	0.63	2.27
bc	5.91	6.28	1	-0.26	0.14	0.48
abc	8.50	8.43	1	0.27	0.14	0.05
Error			8		0.28	

[a] Significance level based on 1 d.f. *$p<0.05$.